Author:
Mountford, Abigail M C

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Synthesis of Novel Substrates to Probe the Specificities of Mupirocin Enzymes

Synthesis of Novel Substrates to Probe the Specificities of Mupirocin Enzymes

Abigail Miranda Clare Mountford

A thesis submitted to the University of Bristol as part of the requirements for award of the degree of Doctor of Philosophy in the Faculty of Science

University of Bristol

School of Chemistry

Cantock's Close

Bristol, BS8 1TS

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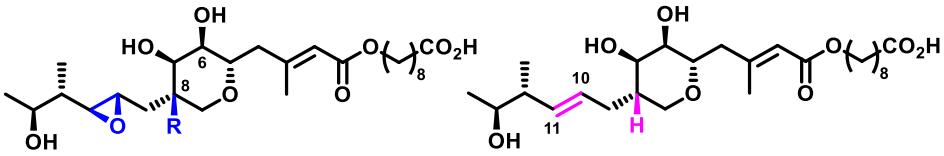
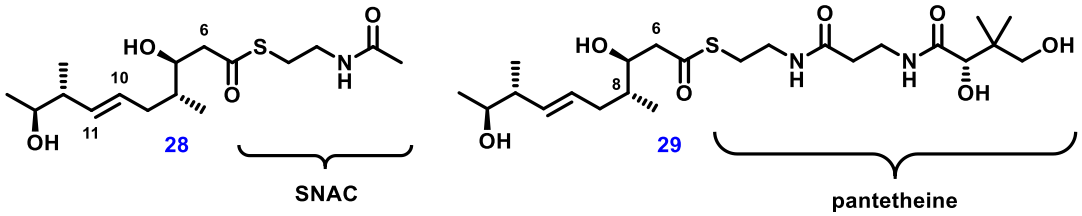
“For with God nothing shall be impossible.” (*KJV*, Luke 1:37)

Abstract

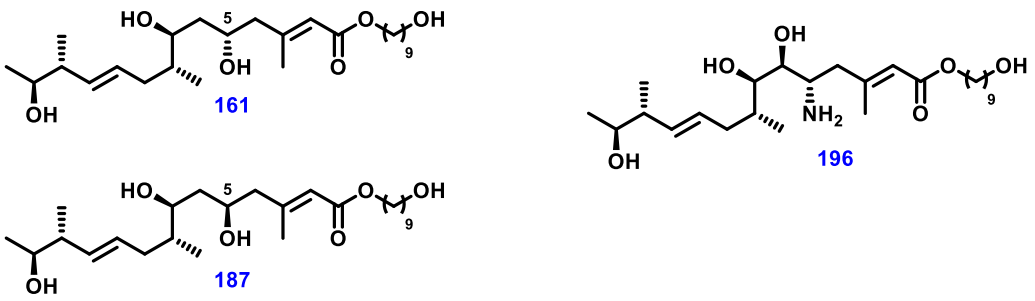
Mupirocin, produced by *Pseudomonas fluorescens*, displays antibiotic activity against a wide range of Gram positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). It is a mixture of pseudomonic acids A, B and C as described in chapter one. Biosynthetic studies have provided an insight into the assembly of the polyketide backbone and post assembly modifications. However, some features of the biosynthetic pathway remain unknown including the timing and mechanism of 6-hydroxylation and the mechanism of tetrahydropyran (THP) ring formation.



Chapter two describes investigations into the gene responsible for 6-hydroxylation, tentatively assigned as *mupA* in mupirocin biosynthesis. Thioester **28** was prepared in 11 steps via a key cross metathesis step to install the 10,11-alkene (pseudomonic acid numbering), followed by an aldol reaction to install the stereocentre at C-7. The synthesis of pantetheinic substrate **29** utilised a hydroboration to install the stereocentre at C-8, followed by a Suzuki cross coupling to establish the *E* alkene. Enzyme assays of these substrates were carried out with MupA, the results of which are discussed herein.



Chapter three describes the synthesis of linear substrates **161** and **187**, and studies towards the preparation of amine **196**, to probe the specificities of MupW and MupZ, the enzymes responsible for the formation of the THP ring. The synthetic route to thioester **29** was adapted to introduce the fatty acid side chain of substrates **161** and **187** via a Mukaiyama aldol reaction. Bioassays of substrates **161** and **187** were carried out to provide insight into both the mechanism of the ring closure and the specificities of MupW and MupZ.



Acknowledgements

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I would like to thank Chris for welcoming me into the group and for giving me such an interesting project. I have learnt so much during my PhD and this wouldn't have been possible without Chris.

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Author's Declaration

The work described in this thesis was carried out in the School of Chemistry, University of Bristol under the supervision of Professor C. L. Willis between September 2016 and December 2019. The work is original, except where indicated by reference in the text, and has not been submitted for any other degrees. The views expressed in the thesis are those of the author and in no way represent of the University of Bristol.

Abigail Miranda Clare Mountford

February 2020

Abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
9-HN	9-hydroxynonanoic acid
Ac	acetyl
ACP	acyl carrier protein
AMR	antimicrobial resistance
Aq.	aqueous
Ar	aryl
AT	acyl transferase
ATP	adenosine triphosphate
Aux	auxiliary
BAIB	bis-acetoxy iodobenzene
bPG	bis-phosphoglyceric acid
c	concentration
cat.	catalytic
CDI	carbonyldiimidazole
CoA	co-enzyme A
conc.	concentrated
CM	cross metathesis
d	doublet
dr	diastereomeric ratio
DCC	*N*, *N*'-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DH	dehydratase
DIBAL	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	*N*, *N*'-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethoxysulfoxide
dppf	diphenylphosphino ferrocene
ee	enantiomeric excess
EDCl	1-ethyl-3-(3-(dimethylaminopropyl)carbodiimide
ER	enoyl reductase
FAD	flavin adenine dinucleotide

FMN	flavin mononucleotide
FMR	flavin monoreductase
GII	Grubbs' second generation catalyst
Glu	glutamine
h	hour
HCS	hydroxymethylglutaryl-CoA synthase
HPLC-MS	high-performance liquid chromatography mass spectrometry
HR-MS	high-resolution mass spectrometry
Hz	Hertz
imid.	imidazole
IR	infrared
J	coupling constant
KR	ketoreductase
KS	ketosynthase
LCMS	liquid chromatography mass spectrometry
LDA	lithium diisopropylamide
LiHMDS	lithium bis(trimethylsilyl)amide
M	molar
*m*CPBA	*meta*-chloroperoxybenzoic acid
mmp	multifunctional modular protein
MOM	methoxymethyl acetal
mp	melting point
MRSA	Methicillin-resistant *Staphylococcus aureus*
N	normality
NAD	nicotinamide adenine dinucleotide
NaHMDS	sodium bis(trimethylsilyl)amide
NHC	N-heterocyclic carbene
NMR	nuclear magnetic resonance
OR	oxidoreductase
ORF	open reading frame
p	*para*
PA	pseudomonic acid
PCC	pyridinium chlorochromate
PDB	protein database

PKS	polyketide synthase
PMB	*para*-methoxy benzyl
PMBDMA	*para*-methoxybenzyl dimethylacetal
PMBTCA	*para*-methoxybenzyl trichloroacetimidate
ppm	parts per million
PPTS	pyridinium *para*-toluene sulfonate
PTSA	para-*t*oluenesulfonic acid
pyr.	pyridine
q	quartet
RMSD	root mean square deviation
RT	room temperature
s	singlet
SET	single electron transfer
SAR	structure activity relationship
sat.	saturated
t	triplet
TBAF	*tetra*-*n*-butylammonium fluoride
TBATB	tetrabutylammonium tribromide
TBS	*tert*-butyl dimethylsilyl
TCA	trichloroacetimidate
TE	thioesterase
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
tert	tertiary
TIM	triosephosphate isomerase
TES	triethylsilyl
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin-layer chromatography
TMS	trimethylsilyl
TPPB	tris-pentafluorophenyl borane
Tyr	tyrosine
VMAR	vinylogous Mukaiyama aldol reaction
WT	wild type

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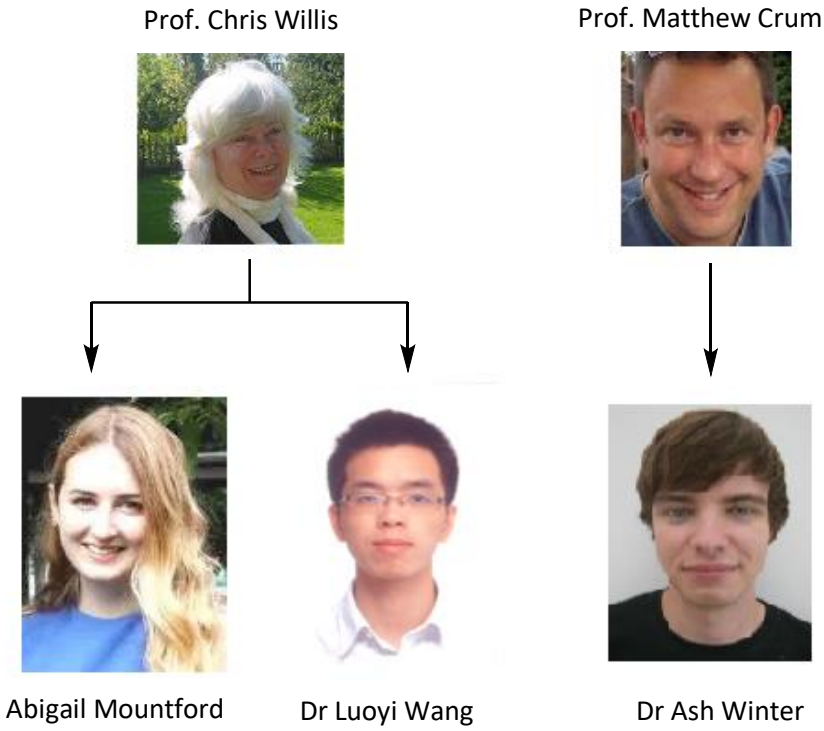
Contributions

This work has been a collaborative project between the Willis Group; Abigail Mountford, responsible for the synthesis of substrates and enzyme assays and Dr Luoyi Wang responsible for gene knockout experiments and enzyme assays, and the Crump group: Dr Ash Winter, responsible for the protein work mentioned in chapter two.

All compounds were synthesised by Abigail Mountford unless otherwise stated. All enzyme assays were carried out jointly by Abigail Mountford and Dr Luoyi Wang. Substrate upgrades and NMR assays described in chapter two were carried out by Dr Ash Winter. All protein crystal images were created by Dr Ash Winter using VMD.



CHAPTER 1: Introduction

1. Introduction

1.1 Antimicrobial Resistance

Antimicrobial resistance (AMR) is established as one of the greatest threats to the global population in the 21st century. A review in 2016 stated that then the current mortality rate from antibiotic resistant diseases was 700,000 cases per year, and was projected to increase to over 10 million per year by 2050.[1]

> "Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi." – WHO (2018).[2]

This rise in AMR is due to several factors, some natural, and some of humanity's own making. Bacteria have evolved to efficiently share genetic material offering a selective advantage, enabling them to quickly overcome new selection pressures,[3-4] though this innate ability has been boosted by several human factors. Over-prescription of antibiotics, lack of awareness as to the correct uses of them, and their wasteful application in agriculture as a preventive rather than a treatment, has stretched the current supply of antibiotics to breaking point. The World Health Organisation (WHO) is backing changes in legislation to improve vaccination rates, provide more funding for research, reduce antibiotic use in agriculture and introduce global surveillance of antibiotic use.[1]

Since the 'golden era' of antibiotic discovery from 1940 to 1960, only a handful of new classes of antibiotics have been discovered,[5] and since 1962 only two new classes of antibiotics have been marketed.[6] In 2017 the BMJ reported that only eight of the 51 antibiotics in clinical trials belonged to novel classes,[7] and most antibiotics currently in development are analogues of existing ones, bearing the same mechanism of action as their parent antibiotic. This is a potentially fatal flaw as it does nothing to combat resistance which has already developed in the bacterial population. Between 1981-2011, 18 of the 26 drugs approved were based on natural products, which is why there has been a drive to develop new drug scaffolds which take inspiration from nature.[8]

Accessing the active components from a natural source can be challenging, especially if only very small amounts are being produced by the bacteria, plants or fungi. In these cases, it may be necessary to synthesise these molecules, which can be laborious and costly. Although published total syntheses often showcase impressive new methodologies, some might argue that it is simpler, more resource friendly, and quicker to re-engineer biosynthetic pathways to either increase the yield of the natural product or enhance its therapeutic properties. Therefore, an understanding of how a natural product is assembled and the genes responsible for each transformation is essential to enabling modification of pre-existing scaffolds in order to produce novel antibiotics based on natural products.

1.2 Polyketides

All living systems rely on a pool of simple organic molecules which they produce via complex metabolic pathways. These metabolites are divided into two key classes; primary metabolites, which are required for the survival of the organism; and secondary metabolites, which confer a selective advantage to the organism.[9]

Polyketides are a large class of secondary metabolites produced by bacteria, fungi and plants which are structurally diverse, ranging from simple polyphenols to complex macrolides.[10] Owing to their structural complexity, polyketides exhibit a wide range of activities including antifungal, antibiotic, anti-parasitic and anti-cancer properties (figure 1). Due to their diversity and utility they are the subject of significant research interest, and make up 20% of all drugs sold worldwide per year.[11]

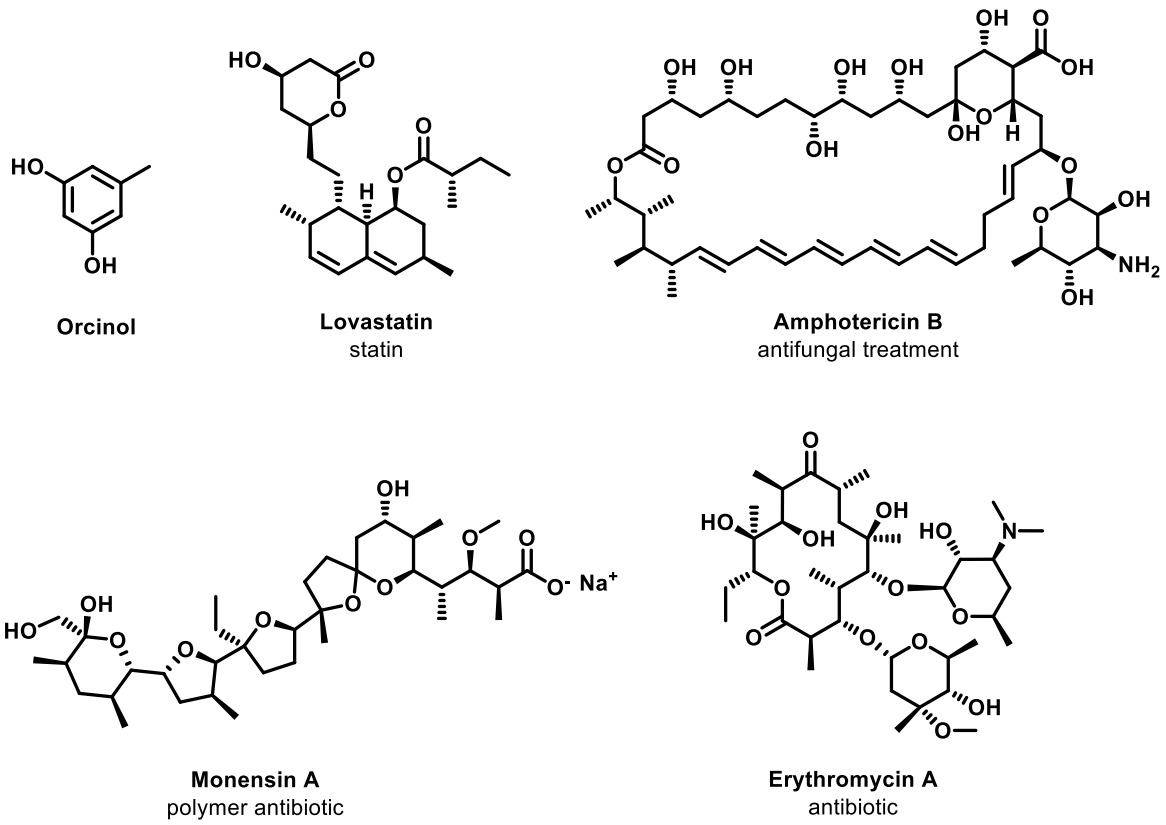
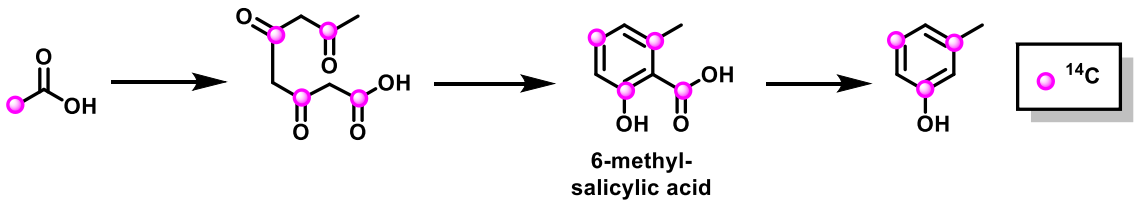


Figure 1. Selected polyketides.

The first polyketide reported was orcinol, which was synthesised by Collie in 1893.[12] He went on to propose a repeating -CH_2CO- 'ketide' unit as the biosynthetic basis of polyphenols in 1907, however it was not until the late 1950s that experimental evidence was provided to support this hypothesis.[13] Through the use of ^{14}C labelling studies, Birch proved that 6-methyl-salicylic acid was produced from a polyketone undergoing cyclisation.[14]



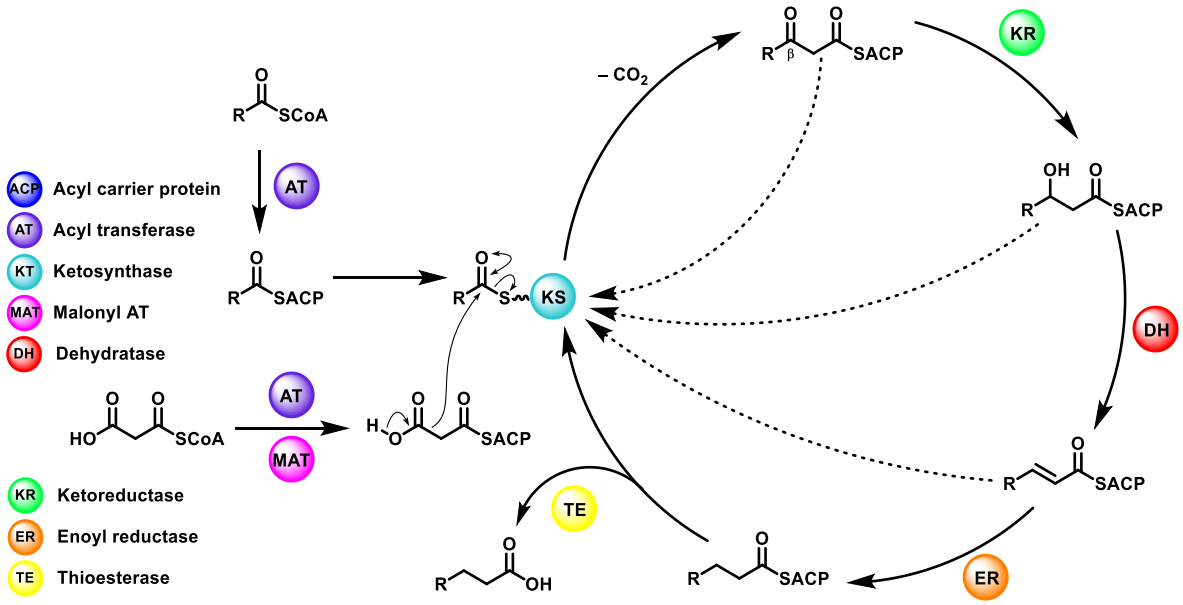
Scheme 1. Isotope labelling studies showing the incorporation of labelled acetate into polyketide 6-methyl-salicylic acid.[14]

Since the seminal work of Collie and Birch,[15] advances in the fields of genetics and genome sequencing have led to a greater understanding of biosynthetic gene clusters and the way in which they function to produce diverse structures.

1.3 Polyketide biosynthesis

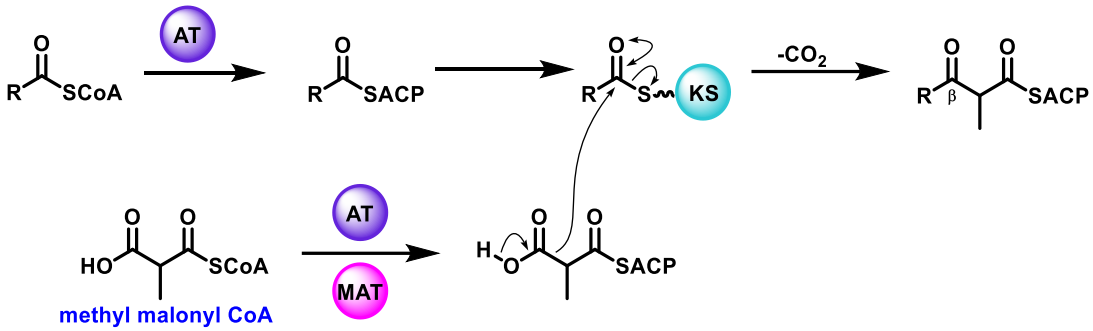
In fatty acid biosynthesis the starter unit is transferred onto an acyl carrier protein (ACP) by an acyl transferase (AT), from which it is transferred onto a ketosynthase (KS) domain. A malonyl CoA unit is loaded onto an ACP *via* a malonyl acetyl transferase (MAT) domain;[16-17] these two units then undergo a decarboxylative Claisen condensation catalysed by the KS. The resulting ACP bound β-ketothioester can be further modified in what is termed β-processing by the action of keto reductase (KR), dehydratase (DH), or enoyl reductase (ER) domains generating the fully reduced carbon chain.[18] Further homologation cycles can take place in order to extend the chain length before the mature fatty acid chain is cleaved from the ACP by a thioesterase (TE) to be released as a free acid.[19]

Polyketide biosynthesis is very similar to fatty acid biosynthesis; however, the reductive processing steps are optional. A typical module selects an extender unit based on the specificity of its acyl AT domain and condenses it with the growing chain using a KS domain. At any time during the β-processing steps, the partially saturated growing chain can undergo further homologation as shown in scheme 2.[20-21] Following hydrolysis, tailoring enzymes can introduce further diversity by carrying out a variety of other useful modifications such as epoxidations, methylations and esterifications.



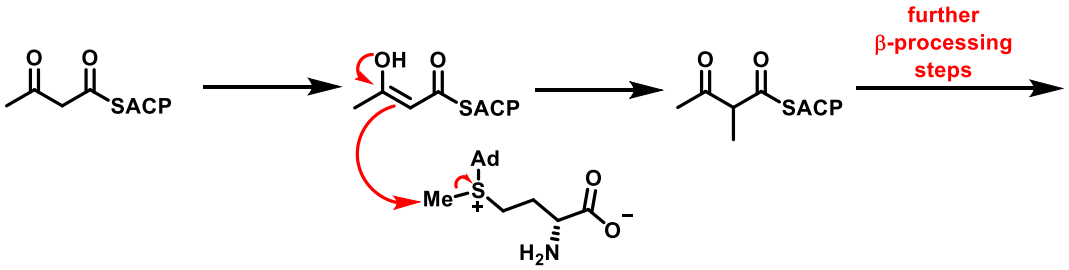
Scheme 2. Biosynthesis of fatty acids and polyketides.

In polyketide biosynthesis the starter unit is often acetate or propionate, though it may be more structurally complex for example benzoate as in the soraphen biosynthesis.[22] This starter unit undergoes a series of two-carbon chain extensions with an extender unit. The most common and simplest extender unit is malonyl CoA, with all others being derived from this structure, for example methyl malonyl CoA which would generate a methyl branch at the α-position in the growing polyketide chain.



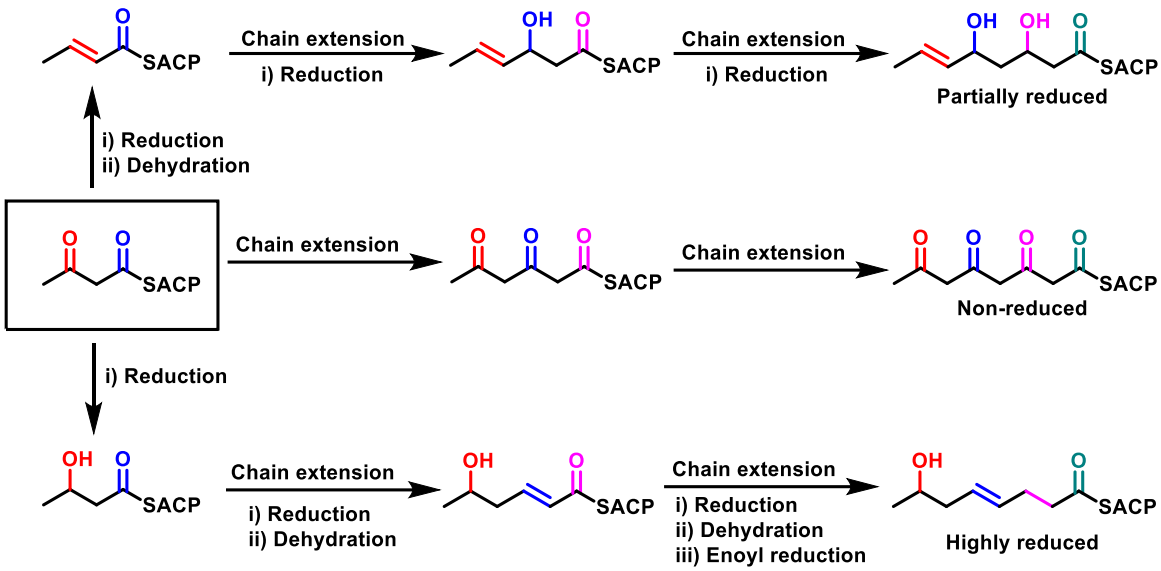
Scheme 3. Methyl malonyl CoA as the extender unit introduces a methyl group at the α-position.

Further modification of the growing polyketide backbone can be achieved by functionalisation at the α-carbons, for example the introduction of methyl groups by the action of S-adenosylmethionine (SAM) (scheme 4). Functionalisation can be also be achieved at the β-position and will be discussed in section 1.6.



Scheme 4. SAM mediated methyl addition.

Unlike fatty acid biosynthesis which only produces fully reduced fatty acid chains, polyketide biosynthesis creates much greater diversity. Depending on how many β-processing steps are carried out, polyketides are classified as highly reduced, partially reduced or non-reduced (scheme 5). Following the Claisen condensation between the starter and extender units, any amount of reductive processing can take place after each chain extension, leading to a vast array of structures with varying levels of oxidation from simple starter units.



Scheme 5. The diversity of structures produced in polyketide synthesis.

There are three distinct classes of PKSs, types I-III. In type I polyketide synthases the domains are arranged linearly[23] and can be subdivided into two categories; iterative, where the domains are covalently linked and can act in multiple cycles; and modular, which are comprised of multi-functional enzymes in which the domains are not repeated and are used only once.[24] Modular PKSs can be further divided into two categories; *cis*-AT in which all the AT domains are contained within the module; and *trans*-AT where catalytic domains are recruited from outside of the module.[25]

1.4 Natural products containing a tetrahydropyran ring

Most drugs on the market today contain at least one heterocyclic system, with Lewell *et al.* reporting that, in 2001, 96% of the 10,000 drugs in development at a major global pharmaceutical company contained a ring system.[26] The importance of these systems has been widely reported, and they have been shown to play a key role in scaffold rigidity, lipophilicity and metabolic stability.[27] Taylor *et al.* reported that in 2014 there were 32 examples of tetrahydropyran (THP) ring containing natural products currently being marketed as small molecule drugs,[27] a selection of which are shown in figure 2.

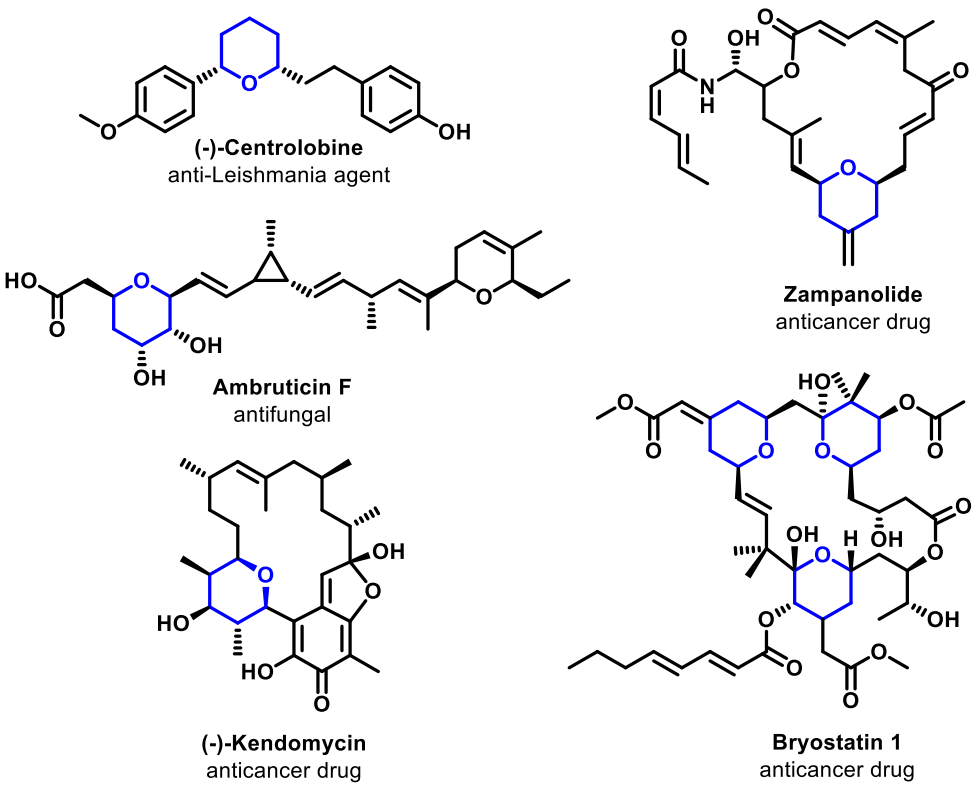
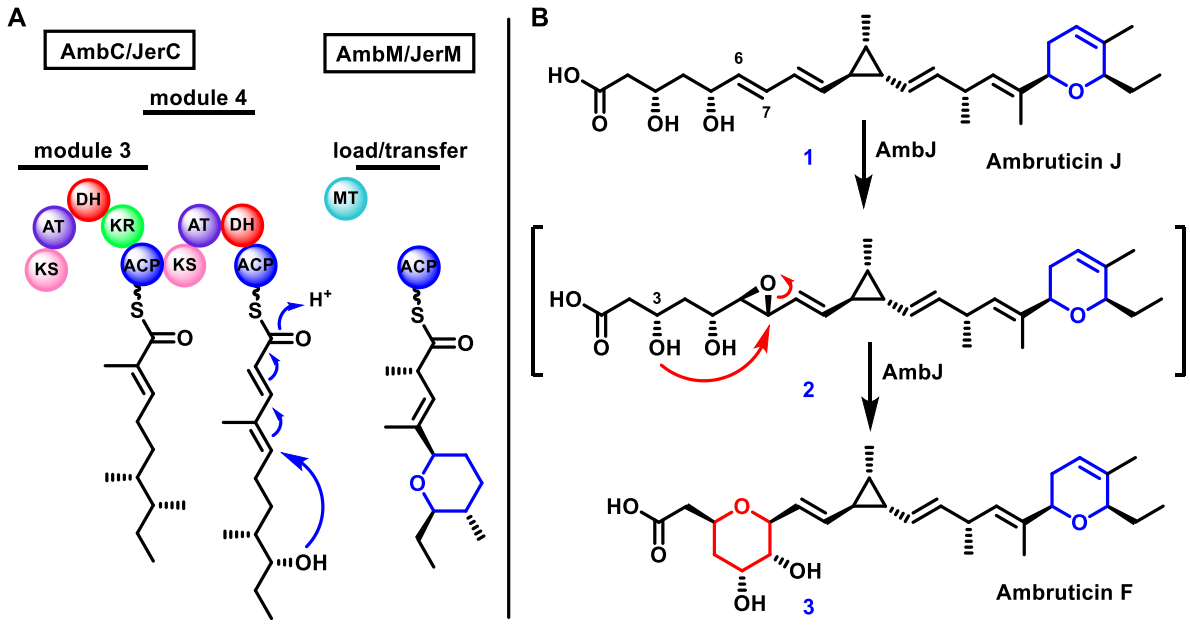


Figure 2. A selection of natural products containing THP rings.

The mechanism of THP formation in natural products often proceeds via an oxa-Michael addition of an alcohol to an unsaturated carboxyl derivative such as in the biosynthesis of the antifungal polyketide ambruticin (scheme 6).[28] The timing and mechanism of this transformation has been elucidated by analysis of the gene cluster and characterisation of metabolites produced by gene knockout experiments (scheme 6).[28] An oxa-1,4-conjugate addition catalysed by the DH domain gives rise to the desired THP, while late stage modification converts this tetrahydropyran to the dihydropyran in ambruticin J **1**.[28-29] Interestingly, the THP ring is formed by a different mechanism, and one that is rarely seen in the biosynthesis of polyketide natural products. Following release of ambruticin J from the PKS, AmbJ, an epoxidase, catalyses the epoxidation of the 6,7-alkene to give **2,** which spontaneously undergoes attack by the hydroxyl at C-3 forming the THP of ambruticin F **3**. This mechanism of THP formation will be discussed further in chapter three.



Scheme 6. An example of two different mechanisms for the formation of THP rings in natural products as seen in ambruticin biosynthesis. **A**: Oxa-Michael mechanism. **B**: Epoxide opening mechanism.[28]

1.5 Mupirocin

Mupirocin is a polyketide derived secondary metabolite produced by *Pseudomonas fluorescens*, a rod shaped bacterium commonly found in the rhizosphere[30] of plant roots, and was first isolated by Ernst Chain's laboratory in 1971 from soil samples taken from Hampstead Heath, London.[31-32] Mupirocin displays biological activity against a wide range of Gram positive bacteria[33] including methicillin-resistant *Staphylococcus aureus* (MRSA)[34] and vancomycin–resistant *Staphylococcus aureus* (VSRA).[35] It comprises a mixture of pseudomonic acids, which are produced by a *trans*-AT type I polyketide synthase and was one of the first antibiotics to be discovered that fell into this category of modular polyketide synthases (PKSs).[36] *Trans*-AT PKSs differ from classic PKSs in that they are not covalently linked to any AT domains. This means that instead, AT activity is provided at each elongation *in trans* by one or more free proteins that are usually encoded in the biosynthetic gene cluster;[20] these act iteratively to introduce a single extender unit in each module.

The major component of mupirocin is pseudomonic acid A (PA-A) **4**, which accounts for over 90% of the mixture (figure 3); Pseudomonic acid B (PA-B) **5** comprises 8% and pseudomonic acid C (PA-C) **6** makes up *ca*. 1%.[37] The general structure of a pseudomonic acid consists of a

monic acid moiety featuring a tetrahydropyran (THP) core bearing a *cis*-diol, which is esterified by 9-hydroxynonanoic acid (9-HN).[38-39]

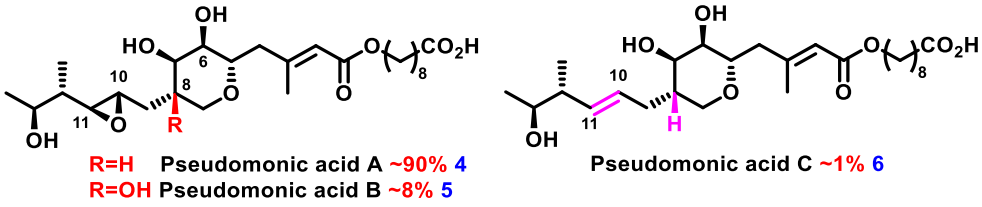


Figure 3. Structures of the pseudomonic acids A-C and percentages found in mupirocin.

In comparison to PA-A, PA-B has an additional hydroxyl group at C-8, whereas PA-C retains the unsubstituted C-8 position but has a 10,11-alkene instead of an epoxide. Mupirocin has been shown to be an effective antibiotic as it inhibits bacterial isoleucyl-transfer RNA synthetase (IleRS), thereby preventing the incorporation of isoleucine into newly synthesised proteins, leaving bacterial cells unable to produce vital cell proteins or assemble their cell walls.[40] Mupirocin was one of the first antibiotics to be discovered with this mechanism of action, and since its discovery only a few isolated cases of bacterial resistance have been reported.[41-42]

The crystal structure of mupirocin binding to its target enzyme IleRS from *Staphylococcus aureus* has been solved (figure 4).[40] The monic acid portion of mupirocin mimics the side chain of isoleucine and so interacts with the isoleucine specific binding pocket of IleRS. The THP ring and the C-1 to C-3 portion[43] mimics adenine and ribose and binds to the ATP binding site.[44] The 9-hydroxynonanoic acid side chain stabilises the complex by binding to a hydrophobic groove in the IleRs, whilst also contributing to the biological properties of mupirocin, as without it the corresponding monic acid is biologically inactive.[44-45]

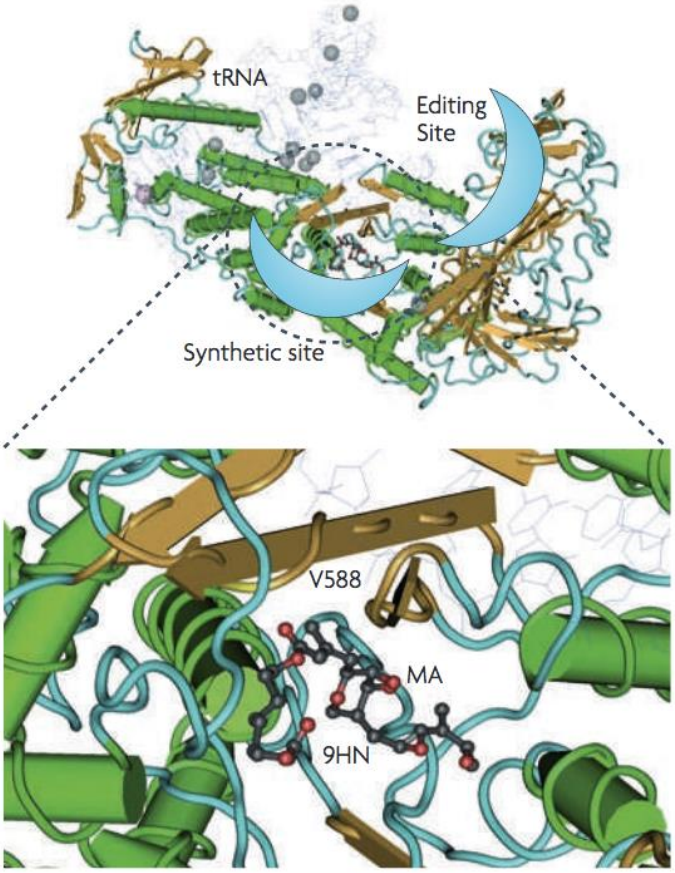
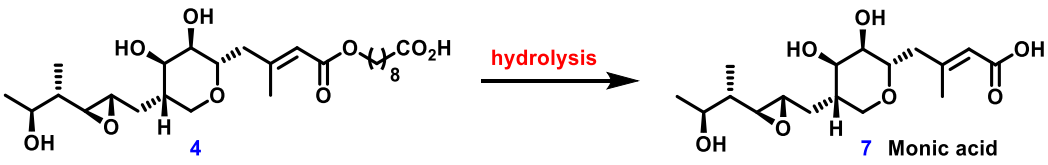


Figure 4. Binding of mupirocin to IleRS from *Staphylococcus aureus*.[30]

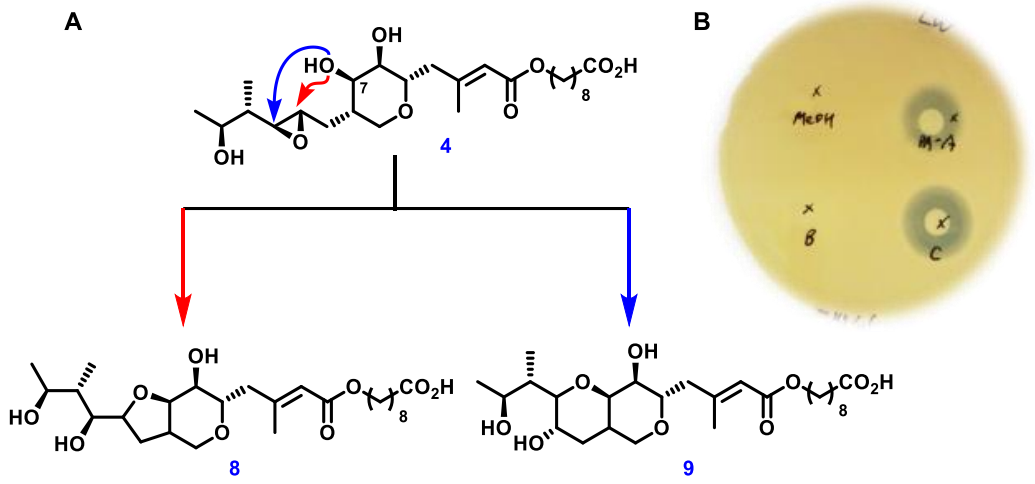
Mupirocin is marketed under the trade name Bactroban® by GlaxoSmithKline and is used for the treatment of minor skin infections such as impetigo,[46] as well as in hospitals to prevent the spread of non-symptomatic MRSA via nasal sprays.[47] Treatment is limited to topical uses, rather than oral or parenteral administration, due hydrolytic instability of the C-1 ester linkage in blood serum, which gives rise to the inactive monic acid A **7** (scheme 7).[48]



Scheme 7. Hydrolysis of PA-A to monic acid.

If used intravenously, the bioavailability is greatly reduced due to its strong binding interactions with blood serum and its activity is limited outside a narrow pH range (pH 5-8) due to the instability of the 10,11-epoxide. In mildly acidic conditions intramolecular attack

of the 7-hydroxyl into the epoxide occurs giving rise to two inactive bicyclic heterocycles **8** and **9**.[49]



Scheme 8. **A**: Intramolecular attack of 7-OH gives rise to two inactive bicyclic compounds.[49] **B**: inhibition zone test showing the activity of the PA-A, PA-B and PA-C.

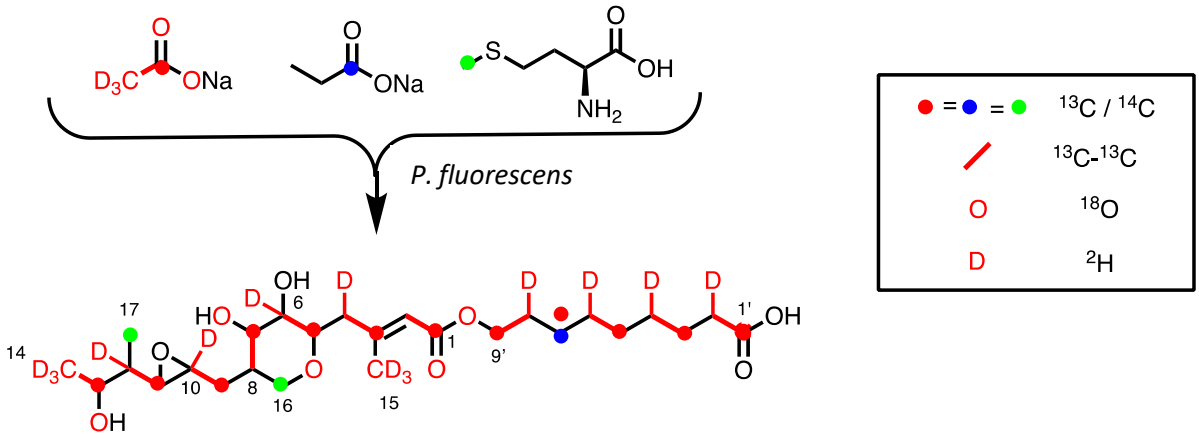
PA-A and PA-C show similar activity against both Gram positive and Gram negative bacteria (scheme 8 B), and it is proposed that PA-C could be used intravenously due to the lack of the unstable 10,11-epoxide. However, PA-C is produced in such low titres that at present its use as a pharmaceutical agent is precluded; it is hoped that a greater understanding of the biosynthetic pathway would allow for genetic modifications offering improved titres.

1.6 Isotopic labelling studies

Since the pioneering work of Birch, isotopic labelling has proved to be a valuable technique to elucidate the biosynthesis of natural products.[50-51] A relevant precursor is prepared incorporating an isotopic label such as carbon-13, oxygen-18 or deuterium, which is then fed to the living organism prior to the production of secondary metabolites. Following incorporation of the labelled substrate, the metabolites of interest are extracted and analysed to determine where the labels have been incorporated in the natural product, providing information about the origin of each of the atoms.

Such labelling studies have been carried out on the pseudomonic acids.[52-53] [1-^{14}C]-, [1-^{13}C]- , [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetates all showed incorporation into pseudomonic acid A, accounting for the carbon backbone (scheme 9).[50] These studies also gave interesting insights into the formation of the ester linkage. Labelling of C-1 and C-9' by [1-^{13}C]-acetate

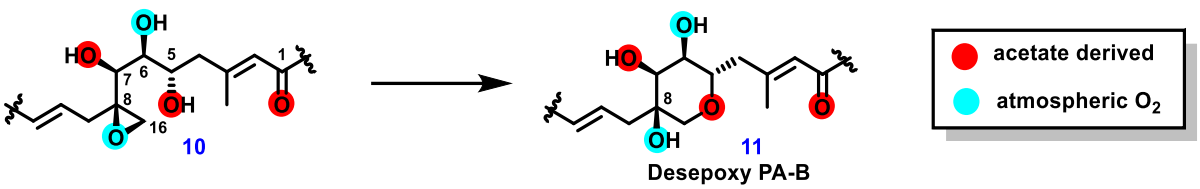
suggested that the 9-hydroxynonanoic acid chain and monic acid are joined by a condensation reaction, and discounted the possibility of the ester being formed through a Baeyer-Villiger type oxidation from a single long chain ketone intermediate.[51]



Scheme 9. Isotopic labelling pattern of pseudomonic acid A.[53]

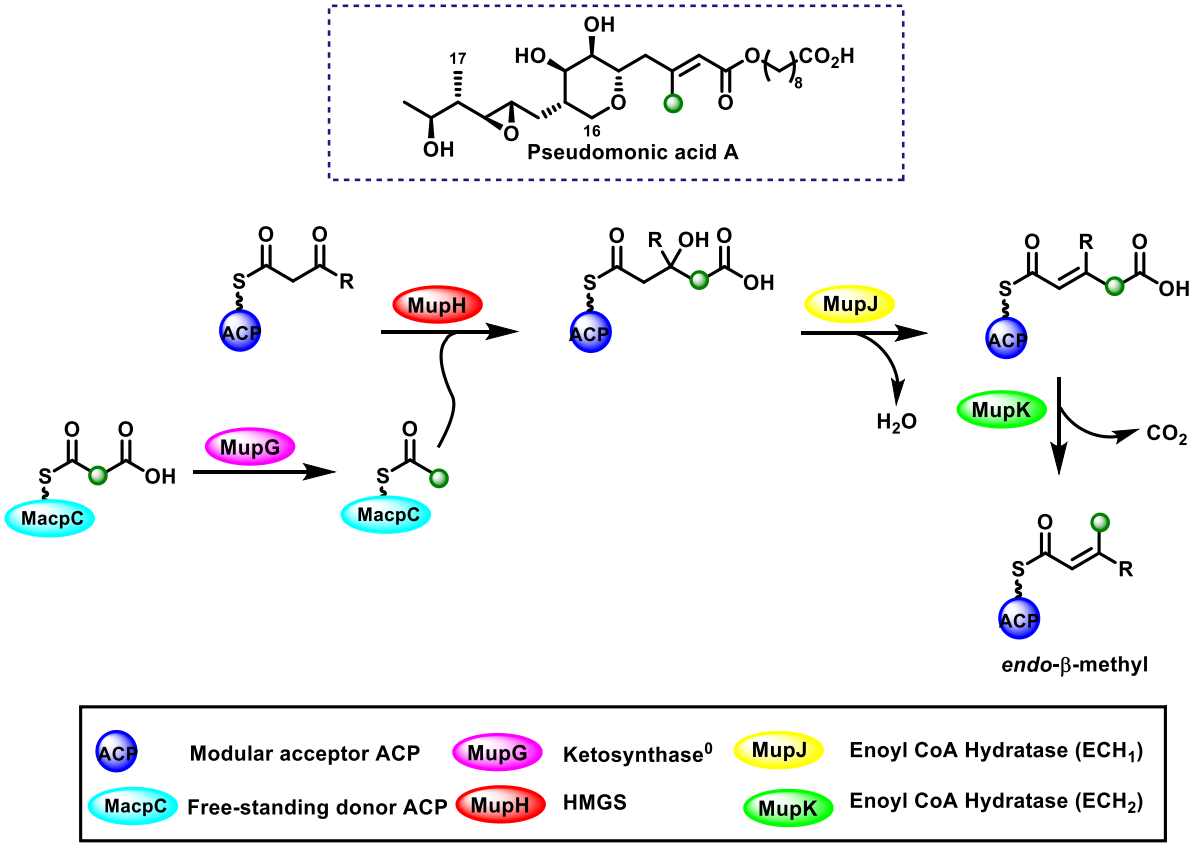
These labelling studies also showed that [1-^{13}C]-propionate was incorporated at C-7', while the 8',9'-acetate unit was not incorporated in the usual 'head to tail' fashion common in polyketides. This suggested that the 9-hydroxynonanoic side chain was formed from two separate biosynthetic units.

Labelling experiments carried out by Martin and Simpson[53] showed that when [1-^{13}C, $^{18}O_2$]-acetate was incorporated, the oxygen atoms on C-1, C-5, C-7 and C-9' were all derived from labelled acetate. This is consistent with the proposed mechanism of THP formation in which the C-5 hydroxyl group attacks the least hindered carbon (C-16) of epoxide **10** (derived from a methyl group) forming the desired THP (scheme 10). The oxygen atoms attached to C-6 and C-8 were presumed to be derived from atmospheric oxygen, with the hydroxyl group at C-8 being removed later in the tailoring steps by MupP, a dehydratase. [36] It is thought that MupA is responsible for the 6-hydroxylation which is discussed in detail in chapter two.



Scheme 10. The origins of the oxygen atoms in the pseudomonic acids.[51]

Labelling studies using L-[Me-^{13}C]-methionine showed that the single carbon branches at C-16 and C-17 are derived from *S*-adenosyl methionine mediated processes as opposed to being derived from methylmalonyl extender units, consistent with the results obtained from the labelled acetate studies.[30, 33, 53] The β-methyl group at C-15 (unlike the α-methyl groups at C-15 and C-17 which are incorporated using an electrophilic methyl source), requires the attack of an 'alkyl' nucleophile at the electrophilic β-position to the ester. Incorporation of [2-^{13}C]-acetate showed that this methyl group was derived from a cleaved acetate unit.[52] Gene cluster analysis identified a cassette of enzymes responsible for this transformation, termed 3-hydroxy-3-methylglutaryl CoA-synthase (HCS). The β-branch arises from the interaction of the HCS cassette with the β-ketothioester bound to an acceptor ACP, the mechanism of which is shown in scheme 11. The cassette consists of a free standing donor ACP (*macpC*), a mutant KS lacking the conserved cysteine required for condensation (*mupG*), a 3-hydroxy-3-methylglutaryl synthase (HMGS, *mupH*) and two enoyl CoA hydratase (ECH) domains (*mupK* and *mupJ*).[30, 36]



Scheme 11. The proposed mechanism for the introduction of the 15-methyl group.[36, 54]

Mupirocin is not the only polyketide to undergo this type of β-branching, and work carried out in the group has identified several polyketides possessing an HCS cassette including kalimantacin A and thiomarinol A. (figure 5).[54-55]

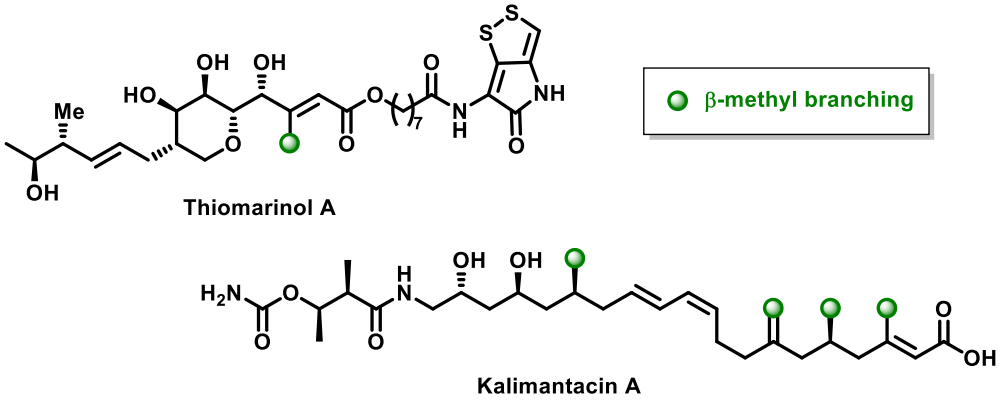
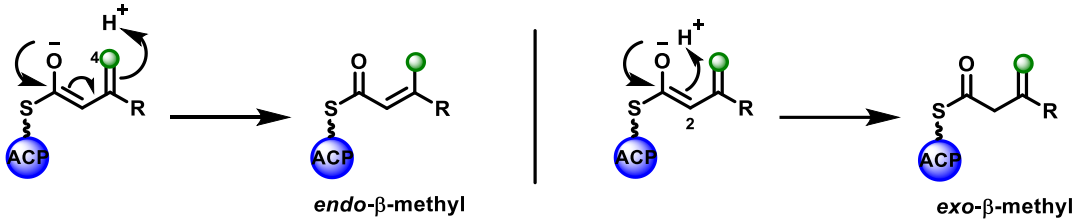


Figure 5. Thiomarinol A and kalimantacin A both contain β-methyl branches.[54-55]

β-methyl branching can be subdivided into two classes: *endo*-β-methyl branching and *exo*-β-methyl branching, depending on the mechanism for reprotonation following decarboxylation.[56] Mupirocin contains an *endo*-β-methyl (α,β-unsaturated β-branch), which is the most common isomer produced by an HCS cassette. This substitution pattern arises from reprotonation of C-4 by the second enoyl coA hydratase domain, which in the case of mupirocin is MupK (scheme 11).[57] If however reprotonation were to occur at C-2, an *exo*-β-methyl (β,γ-unsaturated β-branch) would be formed instead (scheme 12). Kalimanticin is a particularly interesting example of β-branching as it contains both *endo* and *exo*-β-methyl branches which requires high PKS selectivity to ensure the correct β-methyl branch is installed.[55]



Scheme 12. The mechanism of reprotonation to give *endo* or *exo*-β-methyl branches.

1.7 Biosynthesis of the pseudomonic acids

As research into genome sequencing and genetic engineering has become more advanced, gene knockout experiments have enabled the function of genes to be determined more readily.[39, 57] The 74 kb mupirocin gene cluster encodes six modular multifunctional proteins (mmps). The first half of the cluster contains type 1 modular polyketide synthases (PKSs) including multifunctional genes *mmpA* and *mmpD*, the associated *trans*-acyltransferase *mmpC*, *mmpB,* which is a fatty acid synthase, and three single open reading frames (ORFs) *mupZ*, *mupA* and *mupB*.[57]

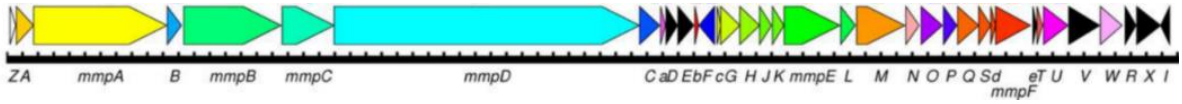


Figure 6. Mupirocin gene cluster organisation.[33]

MmpD and *mmpA* contain four and three modules respectively; each with a ketosynthase domain and an acyl carrier protein domain, and are both responsible for the production of the monic acid portion of mupirocin.[33] The biosynthesis begins with the loading of a malonyl extender unit onto the first ACP of module one catalysed by the AT domain provided in *trans* by *mmpC* (figure 7). There are four condensations carried out by *mmpD* before the growing chain **6** is loaded onto *mmpA*. The isolation of mupiric acid (figure 8) as a result of mutation of the ketoreductase domain in the final module of *mmpD* provides evidence that links *mmpD* to the early stages of monic acid production.[30] The first KS domain of *mmpA* is thought to be a KS^0, an inactive domain, as only six condensations are needed in order to produce the C_{14} backbone.[30] It is used to move the growing chain from *mmpD* on to *mmpA* before two further condensations are carried out to give the monic acid precursor **14**. Deletion of this KS^0 halted the biosynthetic pathway which proves it is essential in the biosynthesis of the pseudomonic acids.[57] The final module of *mmpA* contains twin acyl carrier proteins, each with a unique recognition site for association with the HCS cassette.[30]

An unusual feature of the mupirocin PKS is the absence of any ER domains, which is surprising due to the fact that the 8,9-alkene is reduced in all known pseudomonic acid analogues and has never been observed.[33] Subsequent gene knockout experiments indicated that *mupC,* which encodes a putative dienoyl thioester reductase, acts in *trans* in module 3 to reduce this alkene.[33] This was particularly interesting as it had also been shown

that MupC is involved with the final tailoring steps of the pathway (scheme 13), and these results suggested that MupC could possess the ability to manifest activity on different substrates.[33]

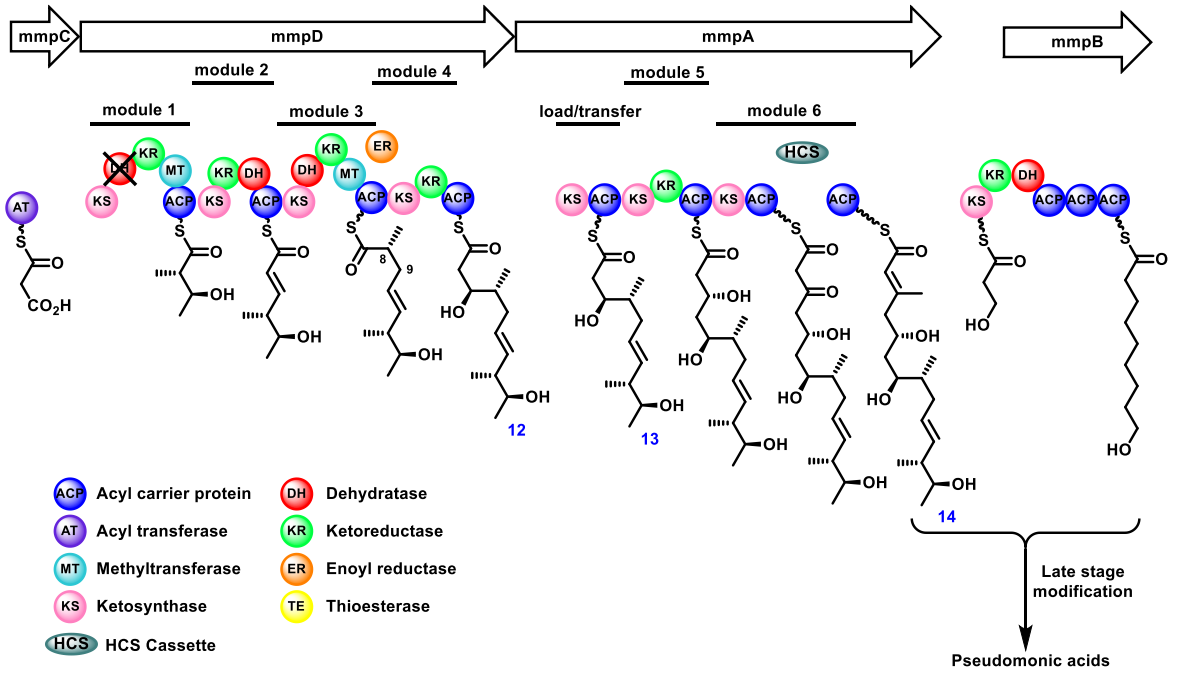


Figure 7. Early stages of the proposed biosynthesis of pseudomonic acids.[30]

The iterative FAS *mmpB* encodes ketosynthase, ketoreductase and dehydrogenase domains and has been shown to be responsible for the biosynthesis of the 9-hydroxynonanoic acid side chain.[58] The starter unit for this for this is proposed to be 3-hydroxypropionate which undergoes three malonate condensations in order to extend the chain. *MmpB* also encodes the only TE in the gene cluster and is therefore likely to control the final steps of the pathway and the release of products.[59] *MmpB* lacks both an AT and ER domain; which are thought to be provided in *trans* by *mmpC* and MupE respectively; and unusually contains three ACP domains.[57] Studies on these ACPs have shown that in-frame deletion of each of the ACPs in turn had no effect on the production of the pseudomonic acids, while deletion of all three abolished production. This shows that the ACP triplet provides a function in parallel but not all are essential.[60] The mechanism of formation of the ester linkage between the thioester of the monic acid portion and 9-HN is still unclear. It has been hypothesised that an esterification takes place between monic acid and 3-hydroxypropionate, with the homologations of the fatty acid chain taking place subsequently, however a three carbon chain on monic acid has never been isolated, which suggests the AcpD which carries this

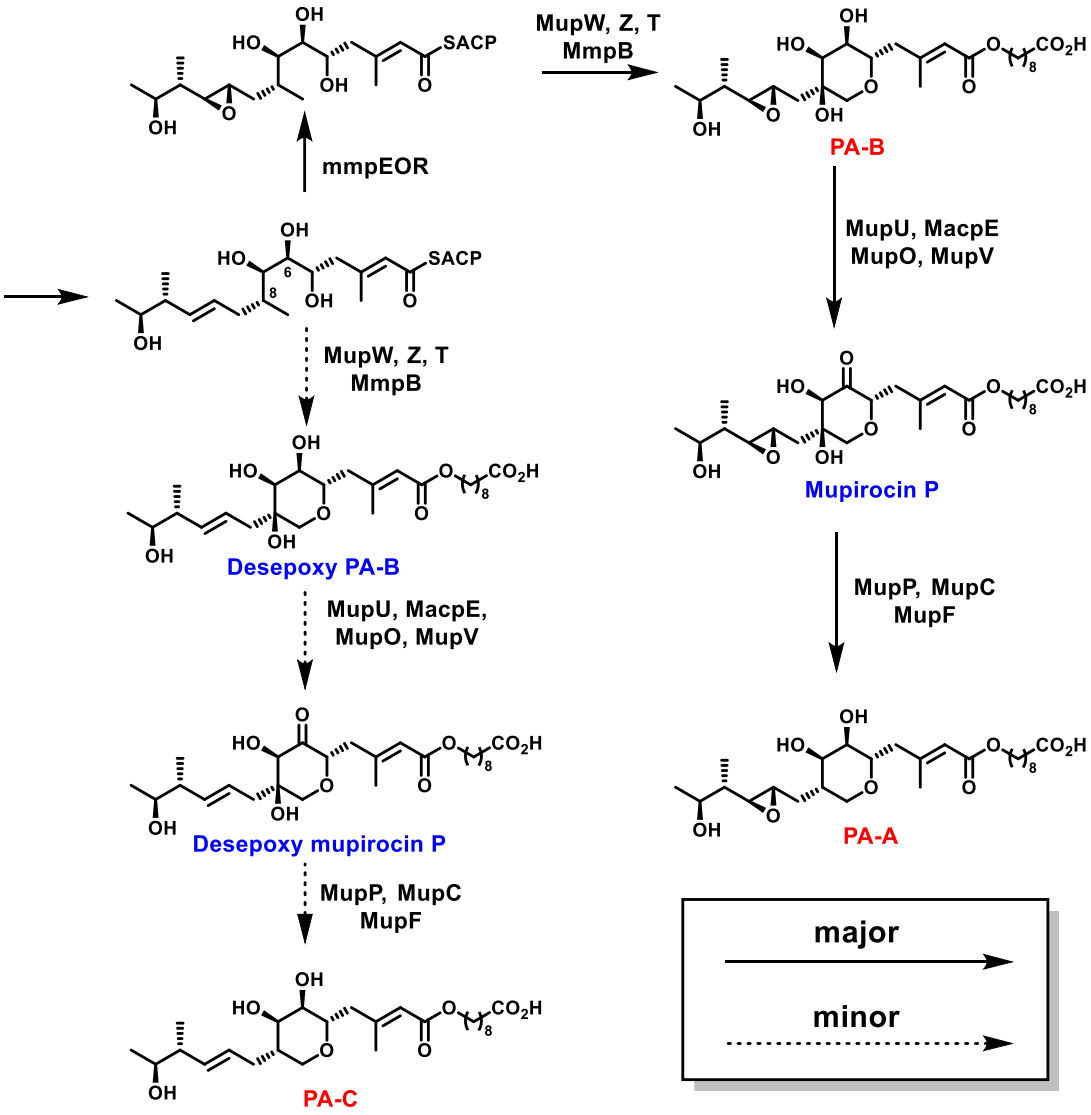
proposed intermediate cannot recognise the esterase. Recent work undertaken in our group has shown that *mmpF* can extend this three-carbon chain to five carbons, however the mechanism for the full chain extension and esterification is still unclear.[61] It has also been suggested that the two portions are produced separately and then esterified, however no accumulation of monic acid or 9-HN has been observed by any gene knockout experiment.

The remaining 27 ORFs are in the second half of the cluster along with smaller PKS-like genes *mmpE* and *mmpF*. Some of these show similarity to type II PKS modules and are known to be tailoring genes, the proposed functions of which are summarised in table 1.[62]

ORF	Deduced functions of the encoded protein.	ORF	Deduced functions of the encoded protein.
mupA	Reduced flavin mononucleotide ($FMNH_2$) oxygenase	***mupL***	Hydrolase
mmpA	PKS (KS, ACP and KR)	***mupM***	Isoleucyl-*t*RNA synthase
mupB	3-Oxo-ACP reductase	***mupN***	Phosphopantetheinyl transferase
mmpB	PKS (FR, DH, KR, ACP, TE)	***mupO***	Cytochrome P450
mmpC	Acyltransferase and ER	***mupP***	DH47
mmpD	PKS (KR, DH, ACP, TE)	***mupQ***	Acyl CoA synthase
mupC	Dienoyl reductase	***mupS***	3-Oxo-acyl carrier protein reductase
macpA	ACP	***macpD***	ACP
mupD	3-Oxo-ACP reductase	***mmpF***	PKS (KS)
mupE	ER	***macpE***	ACP
macpB	ACP	***mupT***	Ferredoxin dioxygenase
mupF	KR	***mupU***	Acyl CoA synthase
macpC	ACP	***mupV***	Oxidoreductase
mupG	3-Oxo-ACP reductase	***mupW***	Rieske type oxygenase
mupH	β-hydroxyl-β-methyl glutarate CoA synthase	***mupR***	Transcriptional activator
mupJ	Enoyl CoA hydratase	***mupX***	Amidase
mupK	Enoyl CoA hydratase	***mupI***	*N*-Acyl homoserine lactone synthase
mmpE	PKS and oxidoreductase	***mupZ***	Epoxide hydrolase

Table 1. The proposed functions of the encoded proteins.[30]

These tailoring enzymes control various processes including the 10,11-epoxidation, as well as 6-hydroxylation and tetrahydropyran ring formation, which will be discussed in detail in this thesis.[19,62] Extensive studies into the late stage modifications in pseudomonic acid biosynthesis have been carried out and have shown that two parallel pathways exist (scheme 13).[36] Classical biosynthetic logic would suggest that PA-C is the precursor to PA-A through epoxidation while PA-B is the downstream product, however this is not the case. Deletion of *mupV*, *mupO*, *mupU*, and *macpE* resulted in the formation of only PA-B with no PA-A detected.[60] When *mupC* was also deleted in addition to one of the four genes mentioned above, the same result was seen, suggesting *mupV*, *mupO*, *mupU*, and *macpE* all act before *mupC,* and therefore that PA-B is the precursor to PA-A as previously suggested but not proven by Mantle *et al*.[63]



Scheme 13. Simplified later stages of the biosynthesis of the pseudomonic acids.[30]

PA-C was shown to be produced via a minor pathway in which *mmpEOR*, an oxidoreductase, does not act and so the epoxide is not installed. However, the following biosynthetic steps are analogous to PA-A in the major pathway: MupP (dehydratase), MupC (dienoyl reductase) and MupF (ketoreductase), which have all been identified and their functions assigned through gene knockout experiments.[36]

Not all of the catalytic functions of the tailoring genes have been so easily assigned due to the so-called 'leaky hosepipe' mechanism.[33] A number of gene knockout and mutation experiments have been shown to produce the same two shunt products: mupiric acid **15** and mupirocin H **16** (figure 8). These are hypothesised to be produced when a mutation occurs that interferes with the formation of 9-HN and/or its esterification onto monic acid. A point mutation of the first ACP in the triplet in *mmpB* (responsible for 9-HN biosynthesis) abolished the production of PA-A, while also producing **15** and **16**.[60] It has been reported that *mupS* and *mupQ* are responsible for the synthesis of the 3-hydroxypropionate precursor to 9-HN, while *mupL* is a potential candidate for attachment of 9-HN to monic acid or its precursor. When these genes were knocked out, again the shunt products **15** and **16** were produced.[33] The conveniently located *mupB*, found between *mmpA* and *mmpB* could allow loading of 3-HN onto the KS of *mmpB* or the monic acid from mmpA-ACP-4 to *mmpB*. These results suggest that halting the biosynthesis of 9-HN means that the final product produced by the PKS is not removed from *mmpA* and therefore not esterified with 9-HN or its precursor. Mutations of the HCS cassette also produced **15** and **16**, which is in accord with the proposal that mutation of the individual functionalities in the cassette impairs the flux of metabolites along the biosynthetic pathway, increasing the dwell time of intermediates at points on the synthase resulting in shunt products being produced.[33] It is likely that these two compounds **15** and **16** are released at chemically labile points as a result of impeding metabolic flux along the pathway, much like the analogy of stepping on a hosepipe. Only when the main flow is blocked do the leaks elsewhere become apparent.

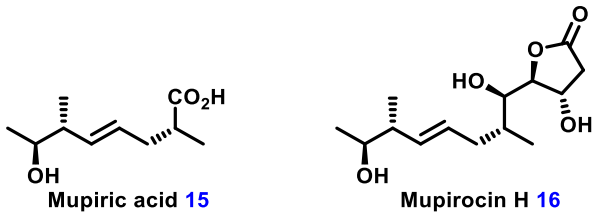


Figure 8. The structures of mupiric acid and mupirocin H.

1.8 Introduction to the 6-hydroxylation in mupirocin biosynthesis

The work in this thesis focuses on two transformations which have yet to be fully elucidated. The first that will be discussed involves investigations into the gene responsible for 6-hydroxylation and the timing of this process in mupirocin biosynthesis.

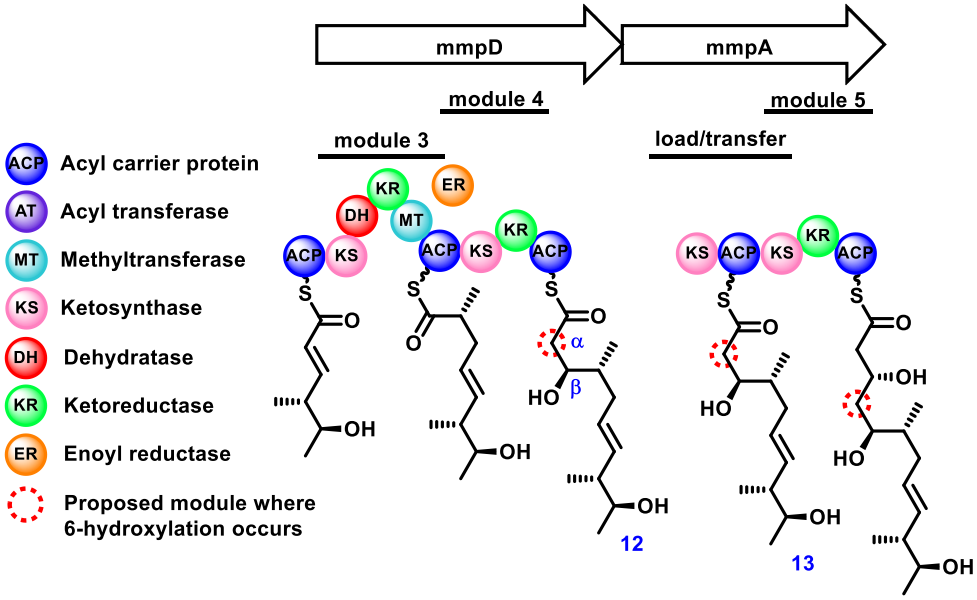
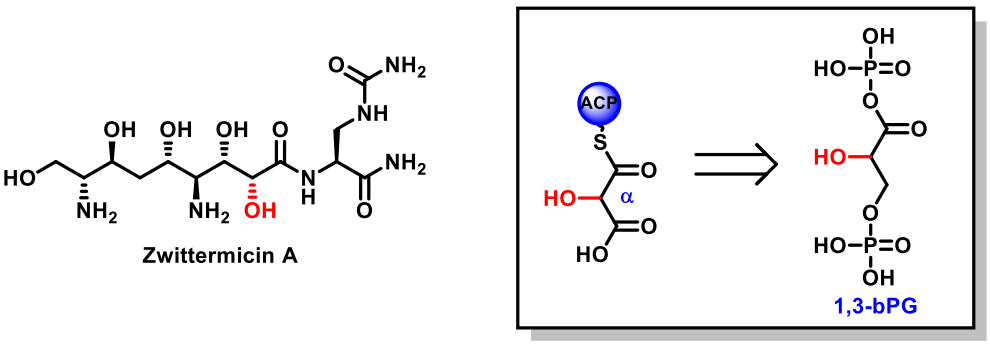


Figure 9. Proposed modules where 6-hydroxylation could occur (red circle).

The introduction of a hydroxyl group is an α-modification which suggests this is being carried out by a tailoring enzyme, the prime candidate for which is MupA, which has no currently confirmed function. We hypothesise that this transformation takes place in the final module of *mmpD* or in the first module of *mmpA* (figure 9).

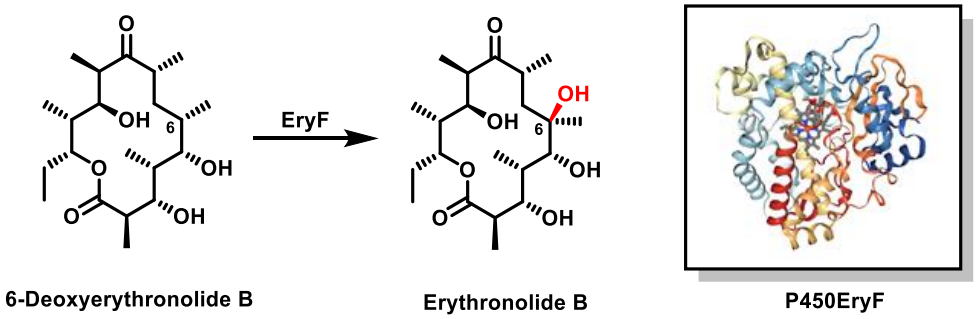
1.8.1 α-Hydroxylases in polyketide biosynthesis

In polyketide biosynthesis, α-hydroxylation has been shown to proceed in a variety of ways, both as part of the PKS machinery and during late stage modification. Incorporation of hydroxy-malonyl ACP bound extender unit is a simple way to functionalise the α-position as demonstrated in the biosynthesis of zwittermicin A, a broad spectrum antibiotic produced by the bacterium *Bacillus cereus* UW85.[64] It is reported that this extender unit is formed from 1,3-bisphosphoglyceric acid (1,3-bPG) (scheme 14), although a number of other mechanisms of formation have been proposed.[64]



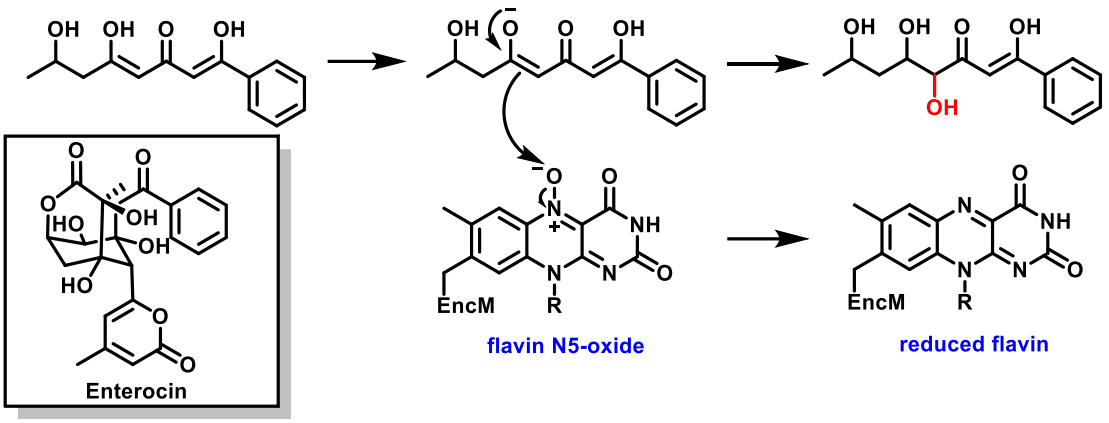
Scheme 14. Zwittermicin A and the hydroxyl-malonyl extender unit formed from 1,3-bPG.[64]

Cytochrome P450 oxygenases are ubiquitous in nature, and are responsible for catalysing the installation of α-hydroxy groups in the biosynthesis of erythromycin,[65] ossamycin[66] and stambomycin.[67] The biosynthesis of erythromycin has been extensively studied[65, 68-70] and the enzyme responsible for the hydroxylation at the 6-position of the macrolide framework has been determined to be the P450 EryF, the structure of which was resolved by x-ray crystallography (scheme 15).[65] As with other P450s, EryF is comprised of a haem prosthetic group embedded between helices, a helical domain and a mixture of β-pleated sheets and coils arranged randomly.[65]



Scheme 15. 6-hydroxylation of 6-deoxyerythronolide B and the enzyme EryF.[65]

EncM is the enzyme responsible for the catalysis of an α-hydroxylation in the enterocin biosynthetic pathway. This process was previously thought to occur via a standard flavin catalysed oxidation, however, studies undertaken by Miyanaga *et al.* proved that the α-hydroxylation was occurring via a novel mechanism utilising a flavin N5-oxide oxygenating species (scheme 16).[71]



Scheme 16. The novel α-hydroxylation in the enterocin biosynthetic pathway catalysed by EncM via a flavin N5-oxide oxygenating species.[71]

The C-7 hydroxyl group present in pederin and closely related antitumor agents, is known to be installed by α-hydroxylation catalysed by flavin-dependent oxygenases at an early stage in their respective biosyntheses.[20, 72-73] It has been shown that PedJ, the enzyme responsible for this transformation in pederin biosynthesis, is a homologue of OnnC and NspB found in the onnamide and nosperin gene clusters respectively (Figure 10 A).[73]

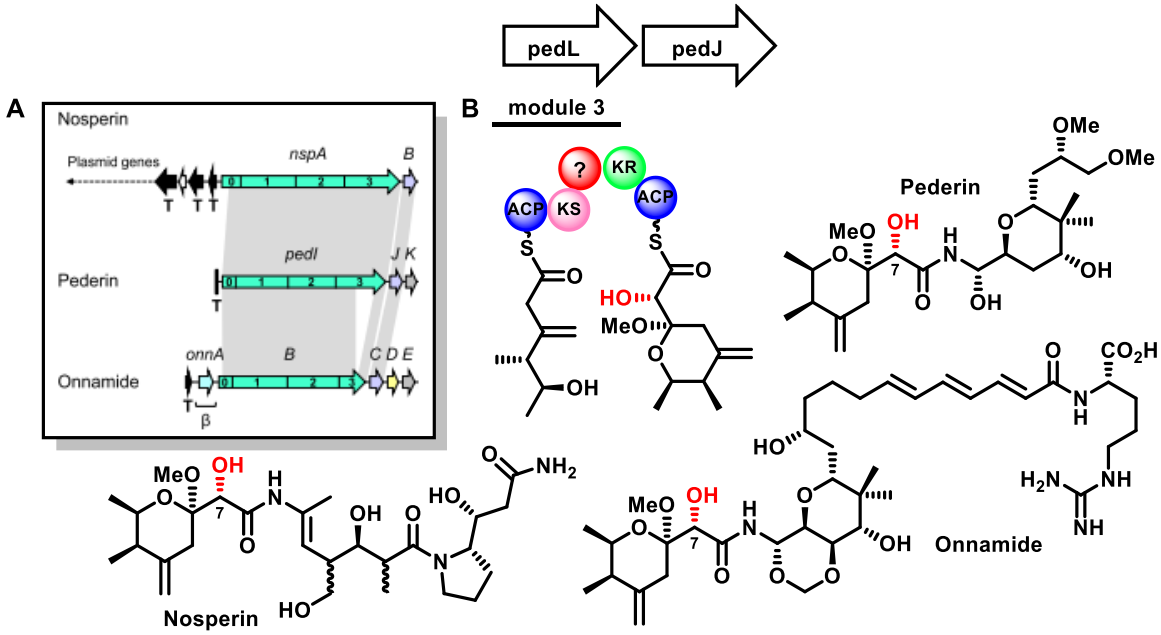
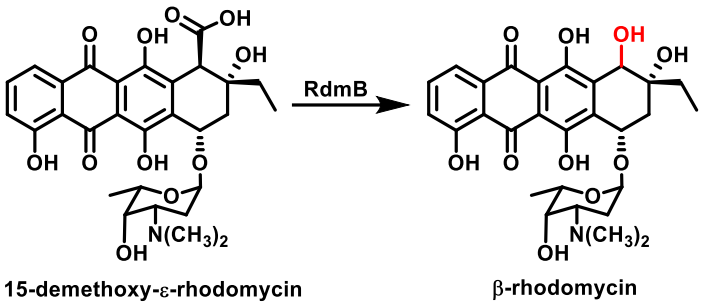


Figure 10. **A**: Gene cluster analysis of nosperin, pederin and onnamide. **B**: α-Hydroxylation catalysed by PedJ in the biosynthesis of pederin.[20, 72-73]

The biosynthesis of the polyketide antibiotic β-rhodomycin involves an esoteric α-hydroxylation catalysed by the *S*-adenosyl-L-methionine-dependent methyltransferase homologue, RdmB. The crystal structure of aclacinomycin-10-hydroxylase (RdmB) revealed

that the enzyme subunit has a fold similar to methyltransferases and binds *S*-adenosyl-L-methionine in an identical manner. RdmB catalyses both hydroxylation and decarboxylation of 15-demethoxy-ε-rhodomycin simultaneously (scheme 17).[74-75]



Scheme 17. α-Hydroxylation catalysed by RdmB.[74-75]

Thiomarinol A, produced by a marine bacterium of the genus *Pseudoalteromonas* is closely related in structure to pseudomonic acid A, however, thiomarinol A possesses a hydroxyl group at C-4, which again arises from α-hydroxylation (figure 11). The enzyme responsible for this transformation has been identified as TmuB which is a member of the non-haem-iron(II)/α-ketoglutarate-dependent dioxygenase superfamily.[76]

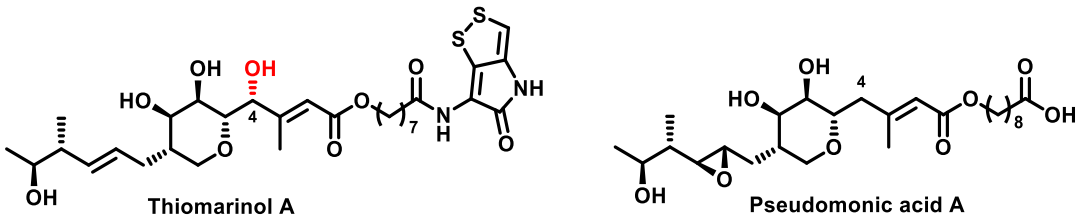
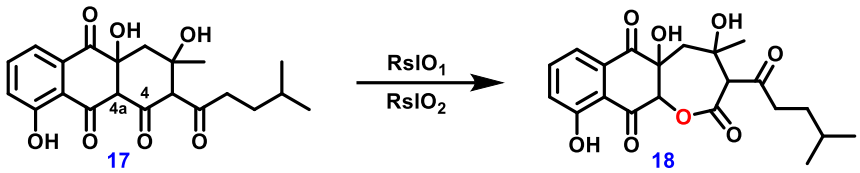


Figure 11. Thiomarinol A and PA-A.[76]

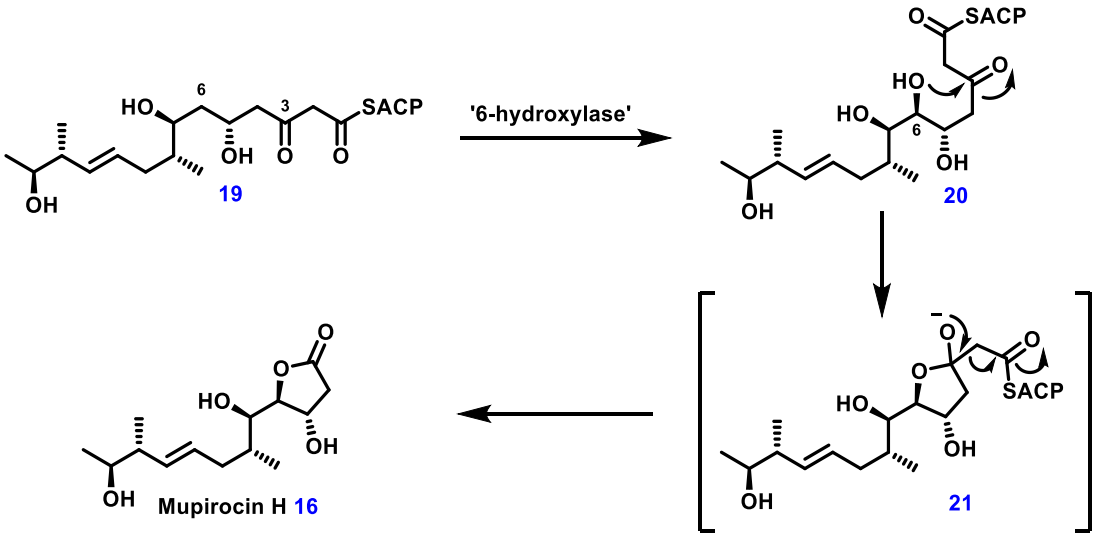
Finally, but not exhaustively, oxygen functionality can be installed at the α-position by luciferase-type monooxygenases. In the biosynthesis of bacterially derived aromatic polyketide rishirilide A, an oxygen atom is inserted into the 4a-4 bond of **17** in a Baeyer-Villiger-type rearrangement.[77] Analysis of the gene cluster showed $RslO_1$ to encode proteins homologous to other luciferase-type oxygenases[78-80] and $RslO_2$ to be a flavin monoreductase which is utilised by $RslO_1$ as a co-factor. The mechanism of oxidations catalysed by luciferase-type monooxygenases will be discussed further in section 1.8.2.



Scheme 18. The installation of an oxygen atom catalysed by $RslO_1$, a luciferase-type oxygenase.[77]

1.8.2 Studies to identify the 6-hydroxylase and the structure of MupA

Previous work in our group involved investigation into the gene responsible for 6-hydroxylation in mupirocin biosynthesis.[33] When gene knockout experiments were carried out with the *P. fluorescens ΔmupH* mutant, mupirocin H was isolated as discussed in section 1.7.[33] The production of this truncated γ-lactone was proposed to be formed via the mechanism shown in scheme 19.



Scheme 19. The proposed mechanism of mupirocin H formation.[33]

It was proposed that a '6-hydroxylase' was acting on thioester **19**, which allowed for intramolecular attack of this 6-OH onto the 3-ketone giving hemiketal **21**, which, following a retro Claisen reaction gives mupirocin H **16**. Previous literature reports have shown that it is possible for enzymes to exhibit dual functionality, for example MupC (section 1.7).[33] Hadatsch *et al*. have reported that if an accumulation of unnatural products from biosynthetic derailment occurs, it is possible for enzymes to act promiscuously,[81] which in the case of *ΔmupH* could have resulted in 6-hydroxylation.

More recent work carried out in our group by Dr Zhongshu Song (unpublished results) showed that when gene knockout experiments were carried out with the *P. fluorescens ΔmupA* mutant, a number of fermentation products lacking the 6-hydroxyl group were isolated, a selection of which are shown in figure 12.

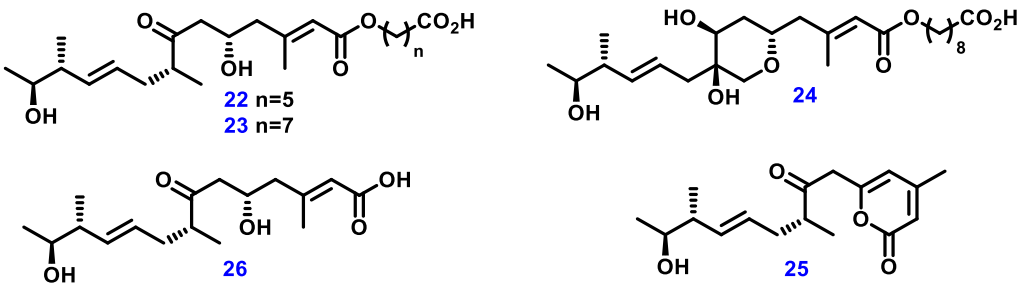


Figure 12. Metabolites isolated from gene knockout experiments with the *ΔmupA* mutant.

Analysis of the mupirocin gene cluster showed MupA to be a monooxygenase which, coupled with the results from the mutant cultures, suggested that MupA was involved with 6-hydroxylation. By comparing the structure of MupA to other known oxygenases, Dr Ash Winter (unpublished results) has shown that MupA is most likely to be a luciferase type monooxygenase. Figure 13 shows the similarity in structure between a flavin dependent monooxygenase, a cytochrome P450, a luciferase and MupA. The smaller the RMSD (root mean square deviation), the closer the structure of the oxygenase is to MupA.

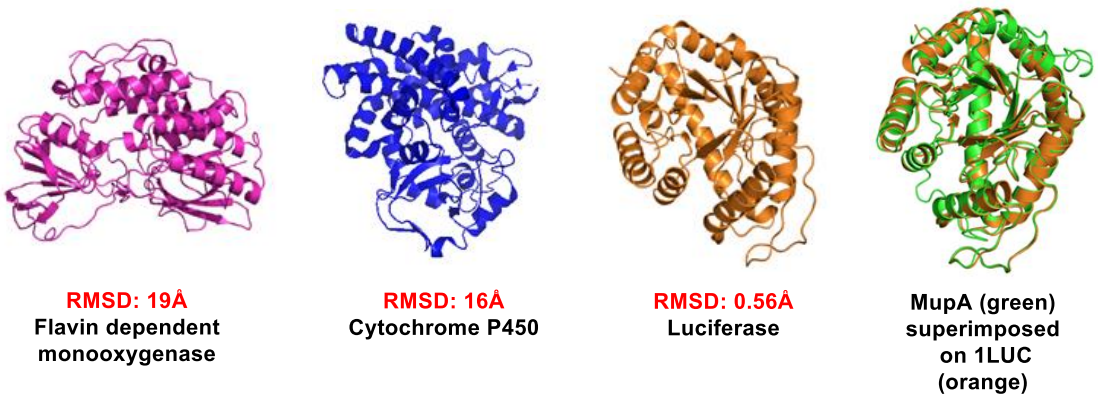


Figure 13. The structures of three different types of oxygenase and the structure of MupA superimposed onto 1LUC a known luciferase type monooxygenase.

By mining the protein database (PDB), it was discovered that a known monooxygenase, 6KET (figure 14 C) had a 47% identity to the structure of MupA and belongs to the luciferase type II family of monooxygenases. Type I luciferase monooxygenases bind the co-factors NADPH and FAD, while type II bind flavin mononucleotide (FMN) as a co-factor and use NADPH as

an electron donor.[82] Type II luciferases contain a triose-phosphate isomerase (TIM) barrel consisting of eight α-helices and eight parallel β-strands that alternate along the peptide backbone.[83] By comparing the structure of MupA to these known luciferase monooxygenases (figure 14) there is a good correlation between the three structures.

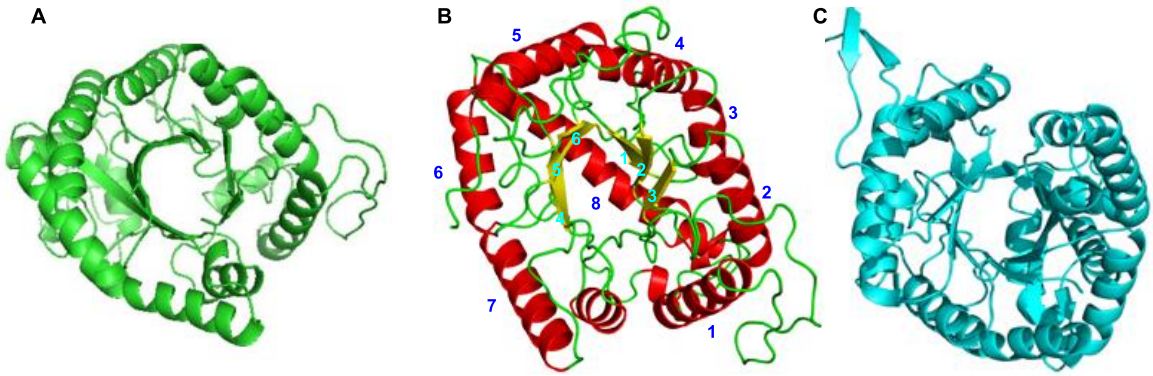
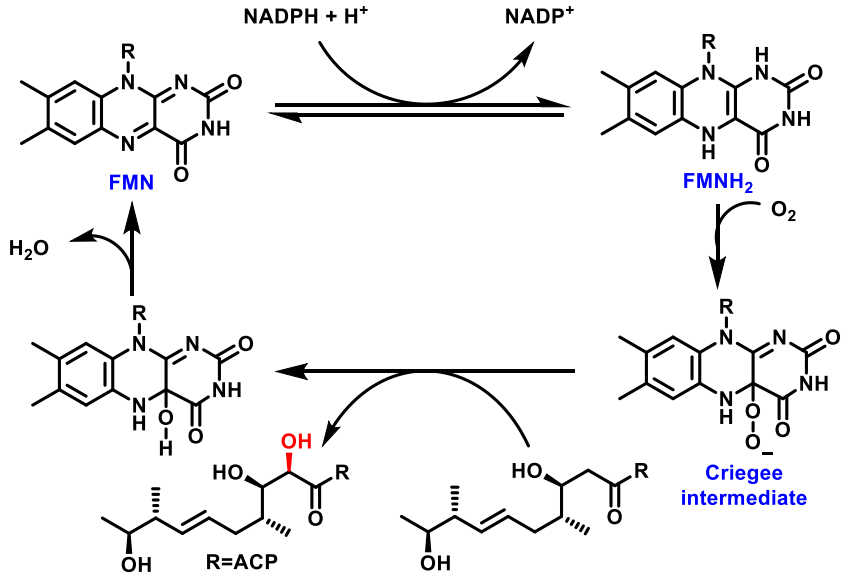


Figure 14. The structure of $RSlO_1$ (**A**), MupA (**B**) and 6KET (**C**).

From this data, it is proposed that MupA is a luciferase type II monooxgenase, which would be unusual as luciferase-type monooxygenases usually act to install oxygen via Baeyer-Villiger-type rearrangements or epoxidations,[84-85] not hydroxylations as in the case of MupA. It is therefore hypothesised that the 6-hydroxylation proceeds via the mechanism shown in scheme 20.



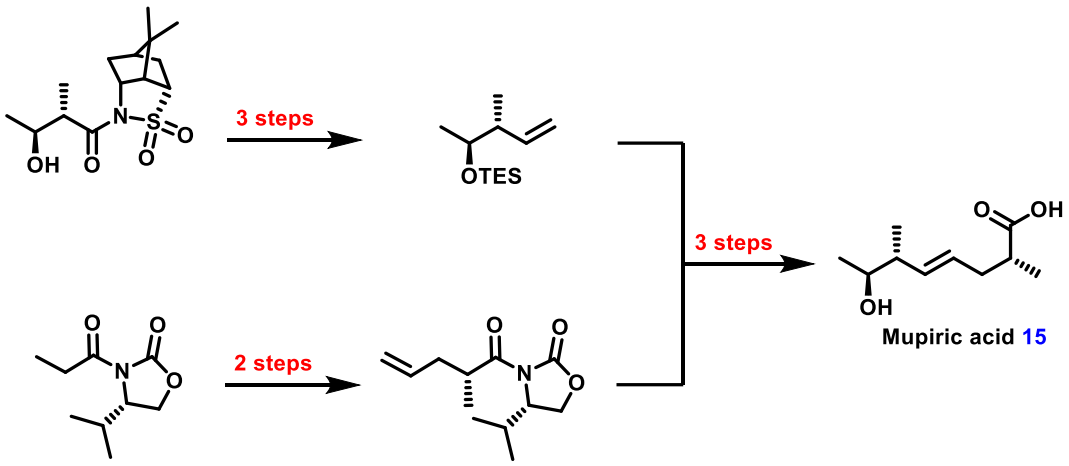
Scheme 20. The proposed mechanism of 6-hydroxylation catalysed by MupA.

The key feature of this mechanism is the reaction of reduced flavin $FMNH_2$ with molecular oxygen to generate the stable flavin-peroxide intermediate (Criegee intermediate).

Following hydroxylation of the substrate, a hydroxy-flavin adduct is produced which is dehydrated to regenerate FMN.

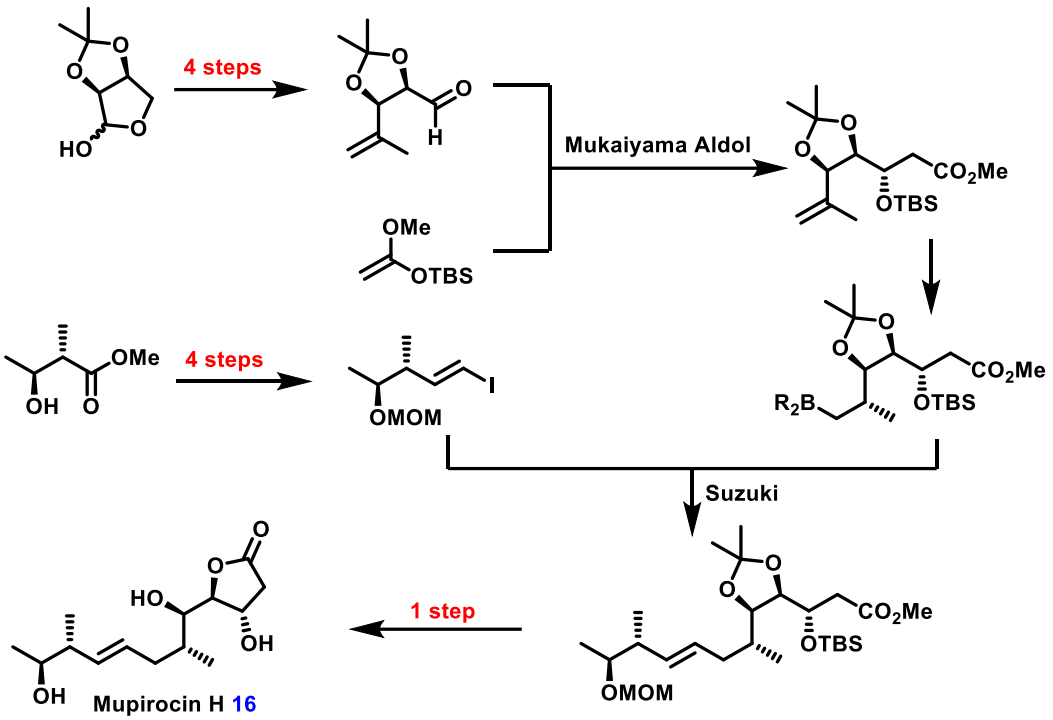
1.9 Previous synthetic strategies to pseudomonic acid based structures

When planning the syntheses discussed in chapter two and three, inspiration was taken from a number of previous total syntheses. The synthesis of mupiric acid **15** was reported by Willis *et al*. in 2008 in six steps (longest linear sequence) utilising a key cross metathesis as shown in scheme 21.[86] Elements of this total synthesis were applied to the syntheses discussed in chapter two.



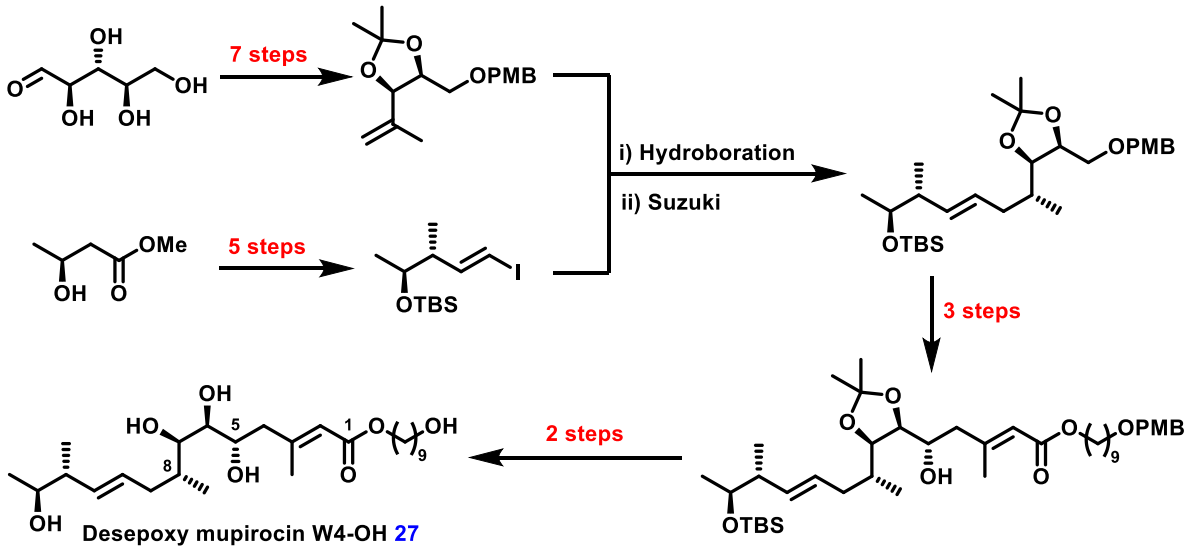
Scheme 21. The synthesis of mupiric acid by Willis *et al*. [86]

The total synthesis of mupirocin H **16** was first reported in 2011 by Chakraborty *et al.* in 19 longest linear steps and 4.96% overall yield utilising a key Julia-Kocienski reaction.[87] In 2012, Willis *et al.* reported an alternative synthesis in 20 steps (longest linear sequence) and 6.2% overall yield, employing ring closing metathesis methodology and a key alkylation step. This was improved upon by Zhao *et al.* in 2013 who reported a total synthesis in seven longest linear steps and 39% overall yield, that utilised a key Suzuki disconnection as shown in scheme 22.[88] The stereoselective hydroboration and subsequent Suzuki reaction were applied to the synthetic route discussed in chapter two.



Scheme 22. Total synthesis of mupirocin H by Zhou *et al*. [88]

An adaption of the synthetic strategy reported by Zhao *et al*. was utilised by Dr Bakar in the total synthesis of desepoxymupirocin W4-OH **27**, which was completed in 14 longest linear steps and in 2.8% overall yield (scheme 23).[89] This synthesis involved a key Mukaiyama aldol reaction to install the fatty acid side chain and will be discussed further in chapter three.



Scheme 23. The synthesis of desepoxy mupirocin W4-OH by Dr Bakar.[89]

CHAPTER 2: MupA – the 6-hydroxylase?

2. Chapter 2

2.1 Project aim

The aim of the first part of the project was to prepare novel substrates **(*S*)-28** and **29** (figure 15) and conduct bioassays with the proposed 6-hydroxylase MupA in order to investigate whether MupA is indeed responsible for this biotransformation. The numbering of carbons in this thesis refers to pseudomonic acid numbering unless otherwise stated. This chapter focuses on structures lacking a 6-hydroxyl group and bearing a side chain which mimics the terminus of an ACP as it is hypothesised this transformation takes place prior to the installation of the fatty acid side chain. The longer the side-chain, the more similar it is to the native ACP and the more likely it is to be recognised and turned over by the enzyme than the analogous carboxylic acid. It was unknown whether MupA would recognise these substrates, and if so, what the phenotypic outcome of these transformations would be.

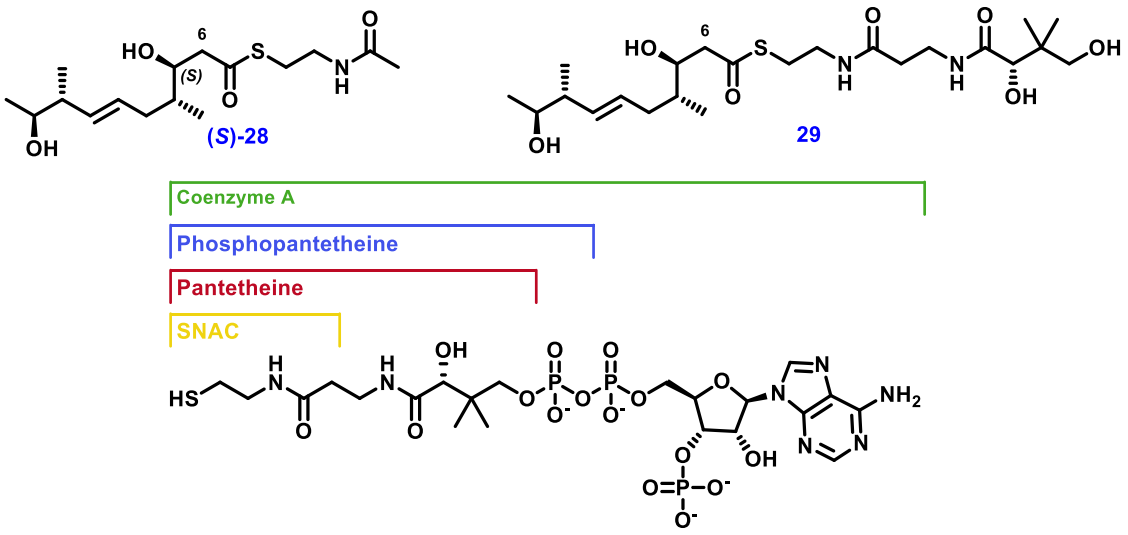
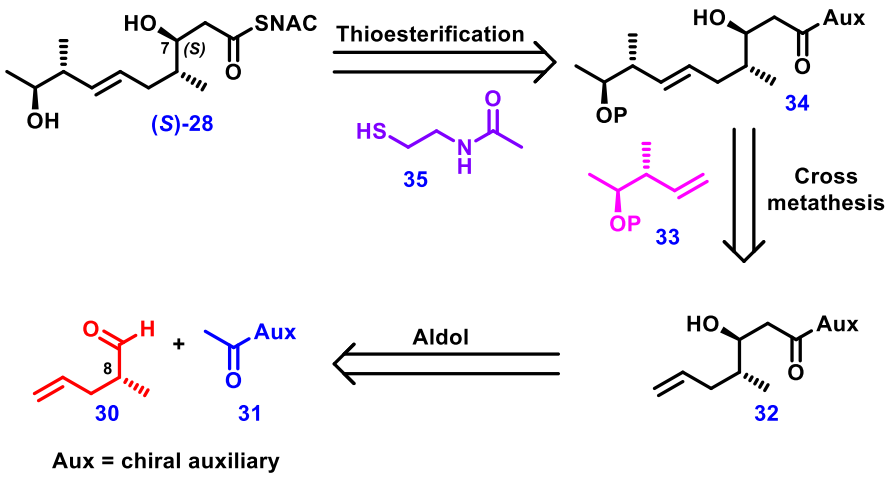


Figure 15. Synthetic targets **(*S*)-28** and **29.**

With this in mind, a retrosynthesis of **(*S*)-28** was proposed whereby aldehyde **30** and acylated chiral auxiliary **31** are coupled under aldol conditions introducing the stereocentre at C-7 and providing a handle for thioesterification (scheme 24). In the next step a cross metathesis would couple alkenes **32** and **33,** creating the *E*-alkene in **34**. In the final step, a thioesterification would introduce the SNAC side chain, which is easily synthesised from the commercially available *N*-cysteamine hydrochloride.[90]



Scheme 24. Retrosynthetic analysis of **(*S*)-28**.

Thioester **(*S*)-28** would then be used in biotransformation studies in *E. coli* expressing MupA to determine whether this enzyme is responsible for the installation of the hydroxyl group at C-6.

2.1.1 Literature research into the key cross metathesis of alkene 32 and 33

Olefin cross metathesis (CM) is a convenient way to take two relatively simple olefins and produce a more complex molecule. Cross metatheses have gained popularity in the last 20 years, due to the development of Grubbs' first-generation **36** and second generation **37** catalysts.

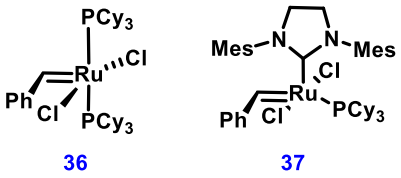
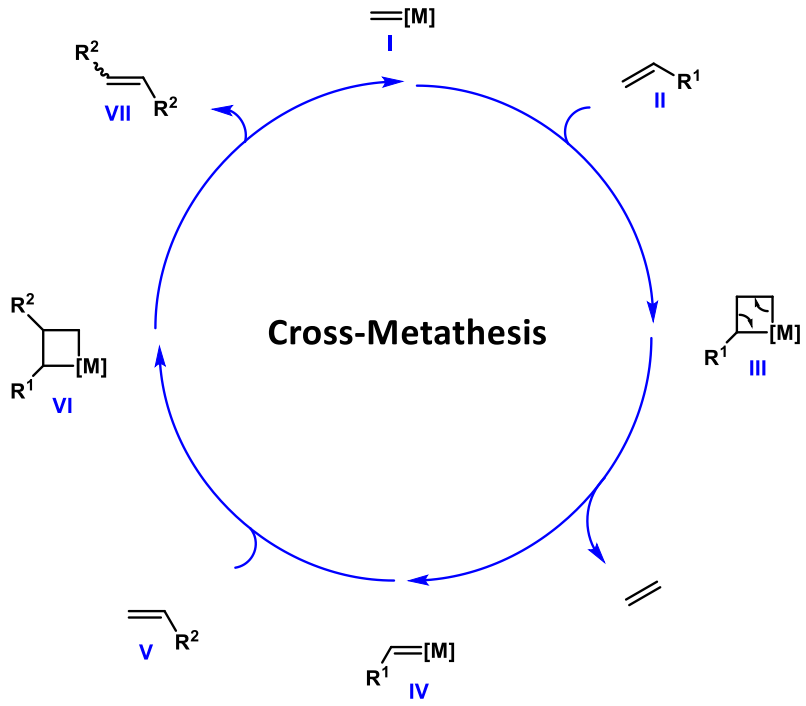


Figure 16. Grubbs' first **36** and second generation **37** catalysts.[91-92]

The replacement of a PCy_3 ligand in **36** by an *N*-heterocyclic carbene (NHC) in **37** leads to a more stable and reactive catalyst due to an enhanced preference for olefin coordination relative to phosphine coordination.[93-95] NHCs are particularly strong σ-donors which show little tendency to dissociate from the metal centre. The substitution of bulky mesityl groups on nitrogen atoms make them able to stabilise the catalytically relevant intermediates against attack by electronic and steric means.[94]

The mechanism of this reaction has been thoroughly studied and has been shown to proceed by initial dissociation of a phosphine ligand to form a 14-electron intermediate **I** shown in scheme 25.[96] A [2+2] cycloaddition between an olefin **II** and this metal carbene intermediate **I** takes place giving a metallacyclobutane intermediate **III**. This metallacycle breaks down in a productive way to form a new olefin and a new alkylidene complex **IV**, or in an unproductive way to regenerate the starting compounds. The alkylidene then reacts with a different olefin **V** to give another metallacyclobutane intermediate **VI** which collapses to give the required cross product **VII** and regenerates the catalyst as shown in scheme 25.[97]

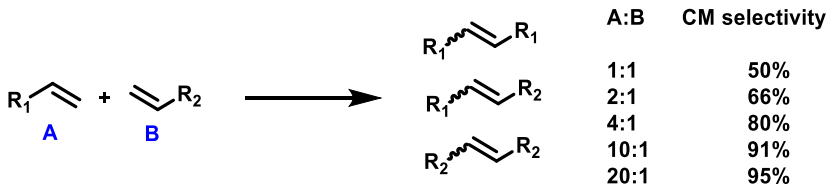


Scheme 25. Catalytic cycle for a cross metathesis.[97]

The selectivity of cross metatheses has been investigated using several different types of alkenes, for example substituted styrenes, 2° and 3° allylic alcohols, and olefins with an α-quaternary centre.[98] Alkenes have been categorised into four types: I, II, III and IV according to Grubbs *et al*.[98] These will not be discussed in detail as the proposed coupling partners in the cross metathesis of alkenes **32** and **33** shown in scheme 24 are terminal olefins and therefore fall into the type I category as defined by Grubbs *et al*.

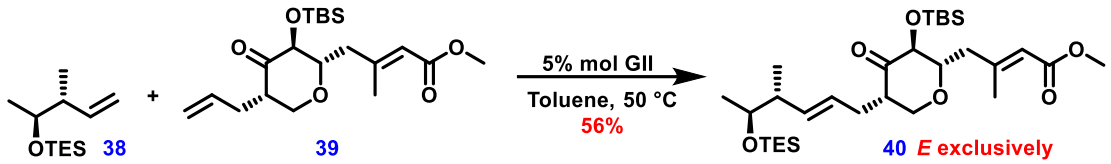
Type I alkenes can rapidly homodimerise and participate in secondary metathesis reactions with type II or III alkenes.[98] Olefin reactivity decreases from type I to type IV as a response to the increase in steric bulk and/or the electron deficiency of their double bonds. Therefore,

careful consideration is needed when coupling any two olefins in the same category, as the kinetic rate towards the homodimer is very similar to that of the heterodimer, which is why usually one olefin is used in excess.[98] Theoretically, up to 20 equivalents of one olefin would provide 95% of the desired product, however, in reality this would be impractical.



Scheme 26. Statistical distribution of products on variation of starting material ratio.[98]

Marko *et al*. reported in the total synthesis of pseudomonic acid C **6** that terminal olefins **38** and **39** were coupled to give the desired *E*- alkene **40** in 56% yield using the Grubbs' second generation catalyst (GII) shown in scheme 27.[99] It was proposed that these conditions could be applied to unsaturated alcohol **32** and alkene **33** to achieve a successful coupling.



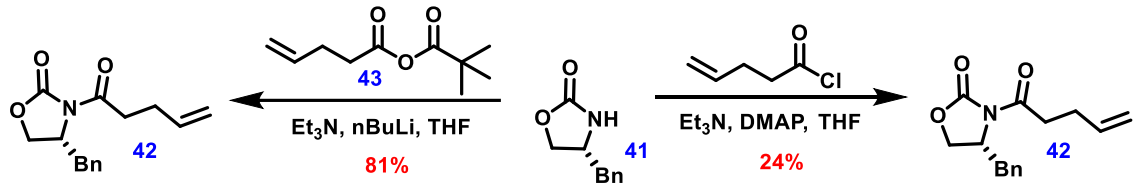
Scheme 27. Coupling of terminal olefins by Marko *et al*.[99]

Both alkene **38** and tetrahydropyran **39** are terminal olefins and so are type I alkenes. Marko *et al.* reported using four equivalents of alkene **38** with one equivalent of **39** in the synthesis shown in scheme 27, so this was considered when planning the synthesis of thioester **(*S*)-28**.

2.2 Results and discussion

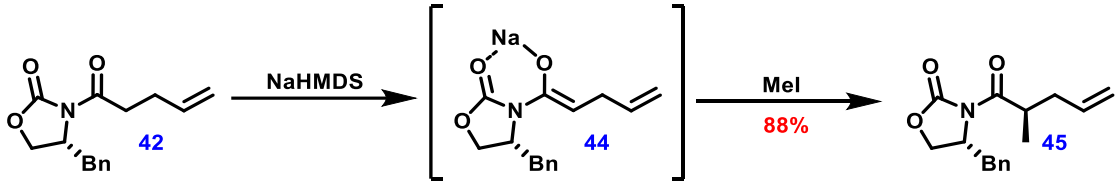
To begin the proposed synthesis of thioester **(*S*)-28**, aldehyde **30** was prepared requiring the installation of the methyl group at C-8 with the correct stereochemistry. The use of a chiral auxiliary was investigated, and although there are many different chiral auxiliaries to choose from,[100] in this case Evans' auxiliary **41** was chosen due to its commercial availability and low cost. Preparation of **42** from auxiliary **41** was investigated using two different methods (scheme 28). The first approach involved the preparation of 4-pentenoyl chloride from the commercially available 4-pentenoic acid which was reacted with Evans' auxiliary **41** in the presence of DMAP and Et_3N as shown in scheme 28. Although this method is reported in the

literature to be high yielding,[101] the best achieved in this study was 24% yield. An alternative method involved formation of mixed anhydride **43** *in situ* before the addition of the Evans' auxiliary **41**[102] which gave **42** in 81% yield.



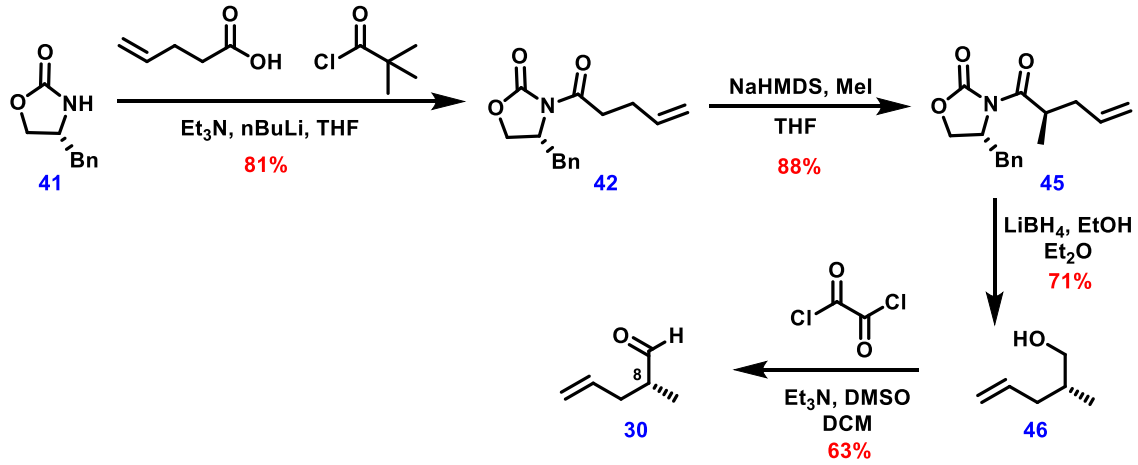
Scheme 28. The two different methods for the synthesis of **42**.

The next step was introduction of the methyl group using methyl iodide and the base NaHMDS. The sodium coordinates to both oxygen atoms to give the (*Z*)-enolate **44** exclusively,[103] holding the transition state rigid while the methyl group is directed to attack from the *Re* face, as the benzyl group is blocking the *Si* face (scheme 29). This reaction gave **45** in 88% yield as a single diastereomer as observed by ^{1}H NMR spectroscopy.



Scheme 29. Coordination of the sodium ion giving the (*Z*)-enolate.

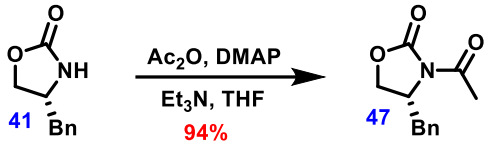
Next the auxiliary was reductively cleaved using $LiBH_4$ to give alcohol **46**, which was oxidised under Swern conditions[104] to give aldehyde **30** in four steps and 32% overall yield as shown in scheme 30.



Scheme 30. Synthesis of aldehyde **30**.

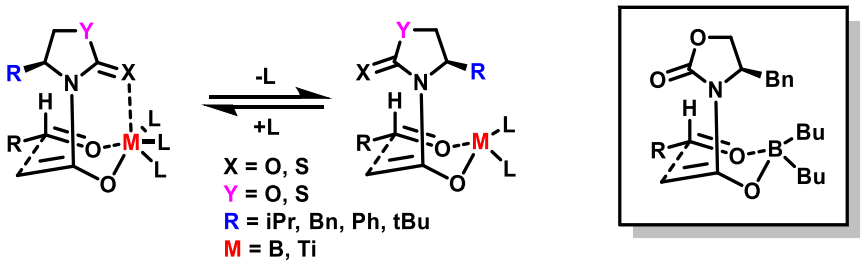
2.2.1 Model studies of the aldol reaction between aldehyde 30 and 47

Acylated chiral auxiliaries have been widely used in aldol reactions[105-106] and to begin with acylated Evans' auxiliary was used. Acylation of **41** under the conditions shown in scheme 31 was straightforward and high yielding, requiring no purification of the product **47**.



Scheme 31. Acylation of Evans' auxiliary.

It has been reported that different Lewis acids give different stereochemical outcomes in aldol reactions with **47**.[107-110] The change in facial selectivity in aldol additions is proposed to be a result of switching mechanistic pathways between chelated and nonchelated transition states due to changes in the metal centre or auxiliary substituent.[105, 107-110]



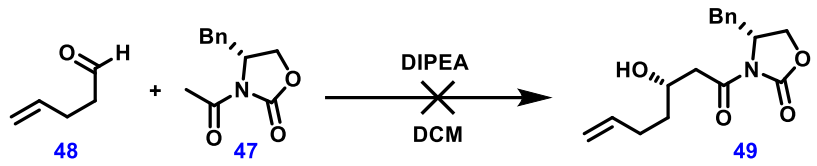
Scheme 32. Chelated vs non-chelated transition states[107] and the 'non-chelated' transition state that occurs when using Bu_2BOTf (right).

To begin, the use of Bu_2BOTf was investigated. This Lewis acid, when used in combination with a chiral auxiliary, to give stereocontrol in a 1,3 fashion with respect to the enolate.[111] Lewis acids containing boron can give a different transition state to those containing titanium. This is because boron cannot expand its octet in order to interact with the lone pair of the carbonyl oxygen in the oxazolidinone. There is also a steric interaction between the auxiliary substituent group (in this case a benzyl group) and the carbon backbone of the aldehyde.[105]

Although the transition state shown in scheme 32 would give rise to a product with opposite stereochemistry to that required for the total synthesis of thioester **(*S*)-28**, the aim of this investigation was to determine whether these conditions could be utilised to give a product

with high *de*. If successful, the opposite enantiomer of the Evans auxiliary could then be used in order to produce the product with the desired stereochemistry. Investigations into this reaction were carried out with the model aldehyde **48**, due to its availability in one step from oxidation of 4-pentenol using pyridinium chlorochromate (PCC).

When aldehyde **48** was reacted with acylated auxiliary **47** using the conditions shown in table 2, the reaction was unsuccessful and gave a complex mixture of products.

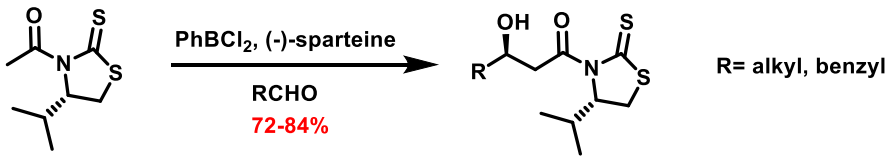


Lewis Acid	L.A Equivalents	Time/ h	Yield/%
$TiCl_4$	2	1.5	0
$TiCl_4$	2	4	0
$Ti(i\text{-}OPr)_4/TiCl_4$	0.2/0.8	2	0
Bu_2BOTf	2	1.5	0

Table 2. The different conditions used to carry out this aldol reaction.

NMR analysis of the crude reaction mixtures showed none of the desired product to have formed, even though the characteristic aldehyde peak had disappeared. After purification neither acylated auxiliary **47** or aldehyde **48** was recovered.

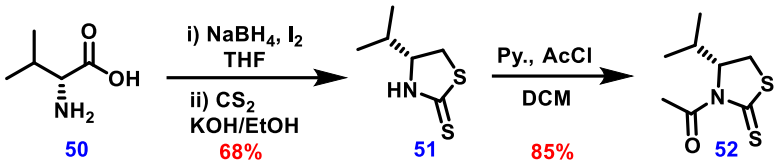
An alternative for stereoselective aldol reactions is the use of *N*-acyl thiazolidinethiones.[107, 112] These acylated auxiliaries have been shown to give products with the desired stereochemistry in high *de*, [99] while also being easily cleaved and thioesterified in one step, something that cannot be achieved with the corresponding oxazolidinone auxiliary.



Scheme 33. Examples of a thiazolidinethione auxiliary being used in aldol reactions.[105]

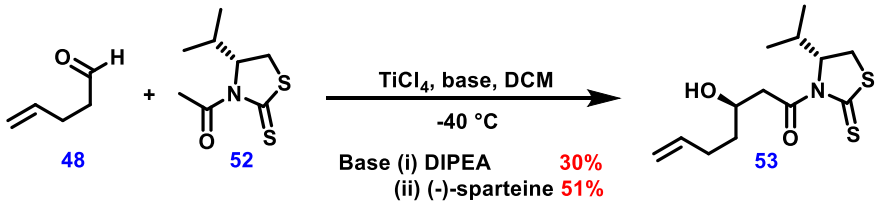
Auxiliary **52** was synthesised in a three-step procedure shown in scheme 34. Reduction of D-valine to D-valinol has been reported using various reagents including $LiAlH_4$ or BH_3-THF.[113] Alternatively an excellent reducing agent for amino acids can be prepared using $NaBH_4$ and

I_2 as first reported by Meyers and co-workers in 1993.[113-114] These reduction conditions have the advantage of being safe and inexpensive, with no racemisation of the product being reported.



Scheme 34. Synthesis of acylated thiazolidinethione auxiliary **52**.

Following reduction of D-valine **50** to D-valinol, the product was refluxed with CS_2 to give thiazolidithione **51** in 68% yield over two steps. Acylation of auxiliary **51** was achieved in 91% yield using acetyl chloride and pyridine in DCM, giving the required substrate **52** to investigate conditions for the aldol reaction with aldehyde **48**.

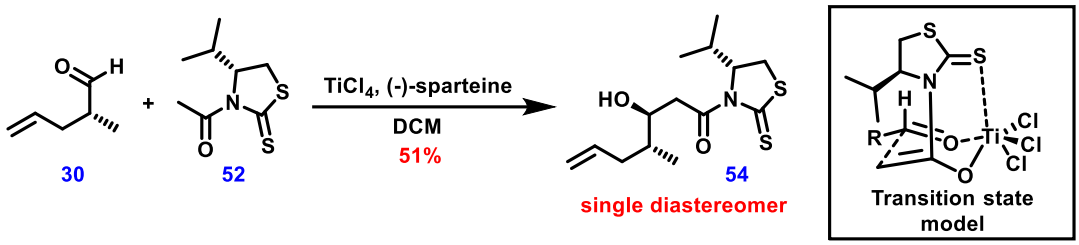


Scheme 35. Aldol reaction using aldehyde **48**.

Two aldol reactions were carried out using neat $TiCl_4$ added dropwise to a stirring solution of auxiliary **52** in DCM. The base (either DIPEA or (-)-sparteine) was then added dropwise and stirred before addition of aldehyde **48** in DCM. $TiCl_4$ was used as literature examples showed the desired stereochemical outcome could be achieved using this Lewis acid.[107] Both reactions were successful, however it was seen that the yield was greatly improved (51% versus 30%) when using (-)-sparteine instead of DIPEA. This effect could be due to the fact that (-)-sparteine is a diamine and so could coordinate to the metal centre in a bidentate manner. Fewer equivalents of the aldehyde would be needed as the base binds to the vacant orbitals on titanium, however this is speculative.[112] (-)-Sparteine has also been shown to dramatically increase the rate of these reactions, although why this happens is still unclear.[115] The asymmetric induction provided by both (-)-sparteine and (+)-sparteine is minimal and (-)-sparteine gives similar diastereoselectivities when either enantiomer of the thiazolidinethione auxiliary is used.[116] The thiocarbonyl of thiazolidinethiones is more nucleophilic than that of the previously used oxazolidinone **47** due to sulfur being larger than

oxygen. The lone pairs on sulfur are more accessible to the Lewis acid leading to a stronger interaction and therefore a more rigid transition state. When thiazolidinethiones are used with chlorotitanium enolates, the reactions are known to proceed via the more rigid 'chelated' transition state as shown in scheme 36.[115]

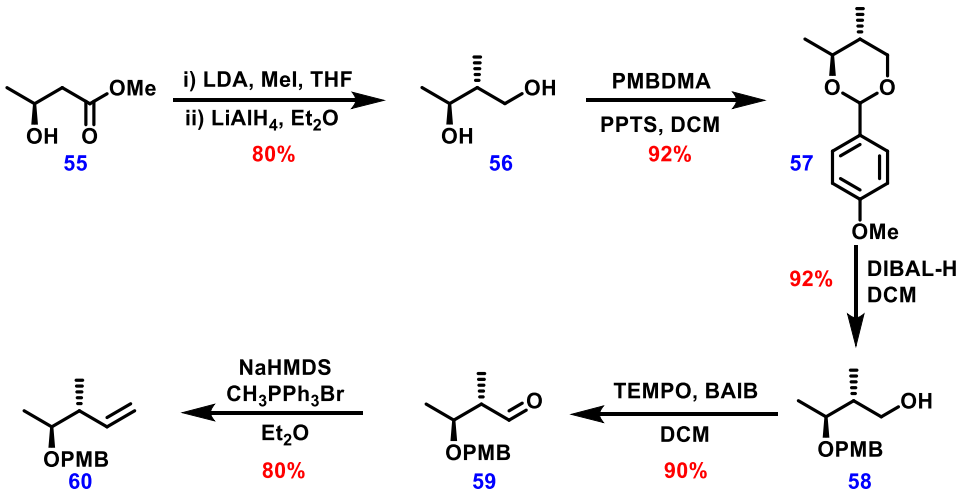
Using the conditions that had been successful in trial reactions (scheme 35), aldehyde **30** and acylated auxiliary **52** were successfully coupled giving alcohol **54** in 51% yield, as a single diastereomer determined by ^{1}H and ^{13}C NMR (scheme 36). The stereochemistry of this product was confirmed by comparison of the spectroscopic data with the literature.[117]



Scheme 36. Aldol reaction of aldehyde **30** and auxiliary **52** giving alcohol **54** as a single diastereomer.

2.2.2 Synthesis of alkene 60 and cross metathesis with 54

The next stage of the synthetic route to **(*S*)-28** required the preparation of alkene **60**, achieved in six steps, for use in cross metathesis chemistry (scheme 37).



Scheme 37. Synthesis of alkene **60**.

In the first step, ester **55** was methylated to give the *anti*-product using LDA and iodomethane. This stereochemistry can be rationalised by the transition state model shown

in figure 17.[118] The lithium cation coordinates to the oxygen of the ester and hydroxyl group forming a 6-membered transition state. The methyl group is then added to the least hindered (*Re*) face, giving the desired *anti* configuration between the methyl and hydroxy groups.[118]

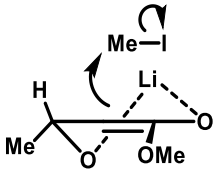
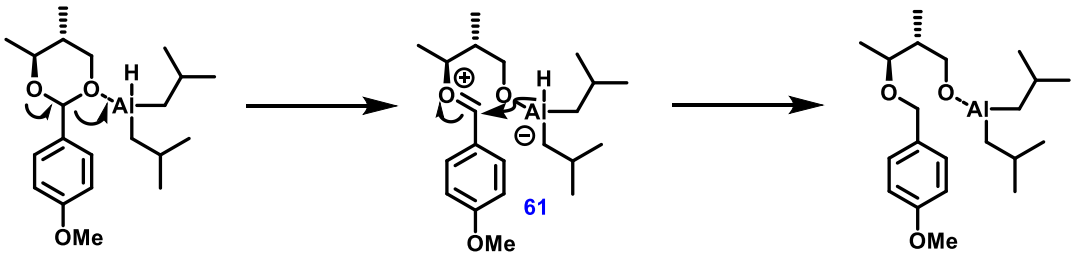


Figure 17. Transition state model for the methylation of **55**.[118]

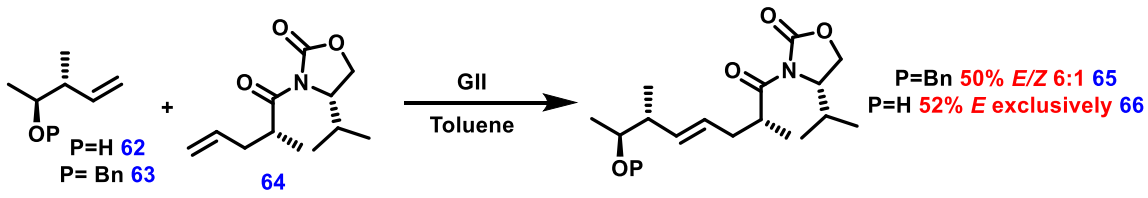
Reduction of this ester with $LiAlH_4$ gave diol **56** which was PMB protected using *para*-methoxybenzene dimethyl acetal to give **57** in 92% yield. Selective reduction of **57** using DIBAL-H gave primary alcohol **58**. The aluminium selectively binds to the least hindered oxygen, which in this case is the primary alcohol (scheme 38). This gives rise to oxocarbenium ion **61**, which is attacked by the hydride, producing the protected secondary alcohol **58**. Work up was simplified by addition of Rochelle's salt which removes the aluminium complex by acting as a ligand, binding to the aluminium and removing it from the product.



Scheme 38. Proposed mechanism of DIBAL-H reduction of **57**.

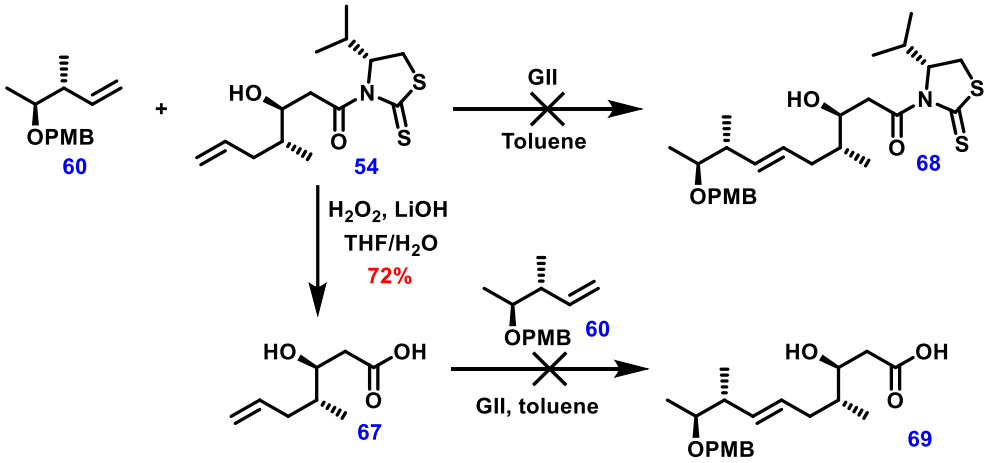
Alcohol **58** was oxidised to aldehyde **59** using TEMPO/BAIB[119] in 85% yield. A Wittig methylenation of aldehyde **59** gave the desired terminal olefin **60** which was the coupling partner for the next stage in the synthesis. Purification of aldehyde **59** by column chromatography was necessary after the oxidation to remove any iodobenzene from the crude reaction mixture as this was inseparable from alkene **60**.

The cross metathesis of **60** and **54** was investigated using conditions previously reported by Marko *et al.* (scheme 27) and used in our group by Mazzetti (scheme 39).



Scheme 39. The cross metathesis carried out by Mazzetti.[120]

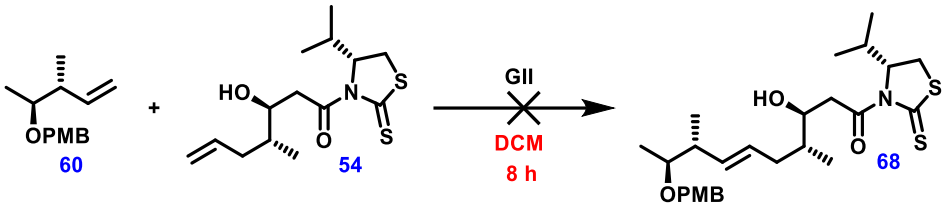
Mazzetti reported that when alkene **63** was used, alkene **65** was formed in 50% yield as a 6:1 ratio of *E/Z* isomers, however when alkene **62** bearing a free alcohol was used, alkene **66** was formed as the *E* isomer exclusively. Using these reaction conditions, the coupling of alkenes **60** and **54** was unsuccessful and starting materials were recovered (scheme 40). It was hypothesised that this coupling was unsuccessful due to the presence of sulfur in the auxiliary, which has been reported to poison ruthenium catalysts.[121] It is known that heteroatoms in close proximity to an olefin can form a chelating ligand on ruthenium, which slows catalyst turnover.[122] To overcome this, the thiazolidithione auxiliary was cleaved using LiOH and H_2O_2 to give acid **67** (scheme 40). Treatment of acid **67** and alkene **60** with GII in toluene again returned starting materials.



Scheme 40. Unsuccessful cross metathesis of terminal olefins **60** and **54**.

Interestingly, the terminal alkene of **60** had partially isomerised to give the more substituted internal alkene, which is a widely reported undesired side reaction that occurs during ruthenium catalysed cross metatheses.[123-127] Nolan *et al*. reported that when GII was used in toluene, the efficiency of the cross coupling was impaired by the tendency of the active species to isomerise the double bonds of the substrate, leading to more highly substituted alkenes which are stabilised by hyperconjugation.[128-129] This isomerisation can also be the

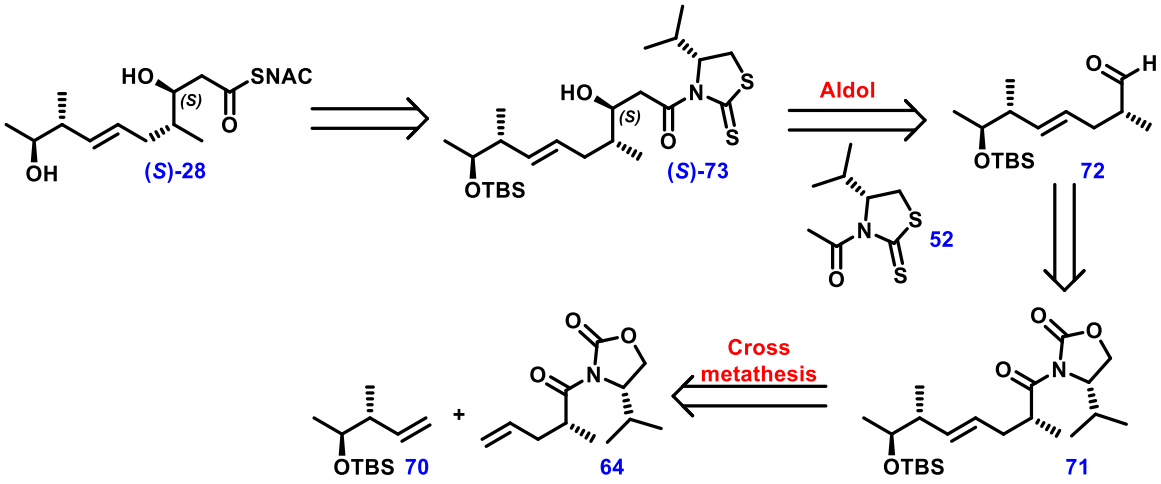
result of decomposition of the ruthenium catalyst due to reaction times of over 24 h,[130] hence the cross metathesis of alkenes **60** and **54** was repeated for a shorter reaction time (8 h) and in a different solvent (DCM), however isomerised starting material was still observed by ^{1}H NMR and none of the required coupled product **68** was detected (scheme 41).



Scheme 41. Attempted cross metathesis in DCM with a shorter reaction time.

2.3 Revised Synthetic Approach to (*S*)-28

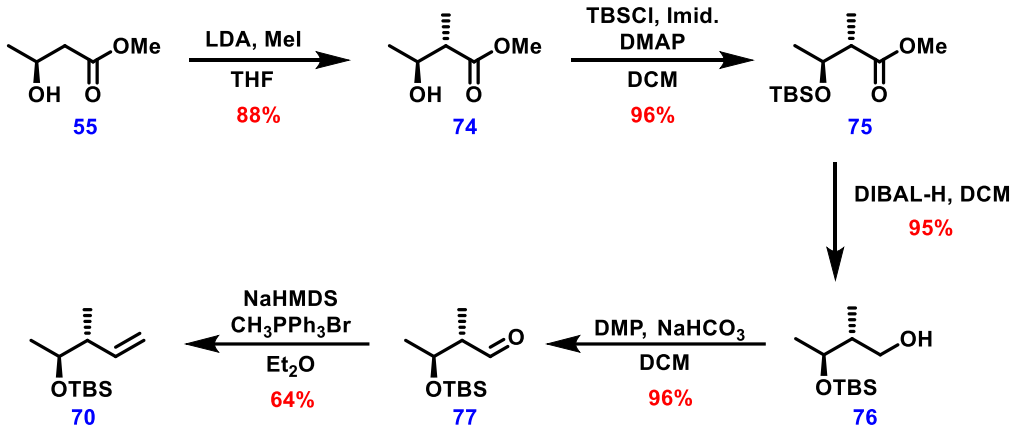
Based on the results to date a new synthetic approach was designed which still incorporated the two key disconnections a stereoselective aldol and cross metathesis, however in reverse order (scheme 42). As thiazolidithione auxiliary based alkene **54** had been an unsuitable coupling partner for cross metathesis, it was decided to change the protecting group strategy and to prepare alkenes **70** and **64** for the key cross metatheses step.



Scheme 42. Revised retrosynthesis of **(*S*)-28**.

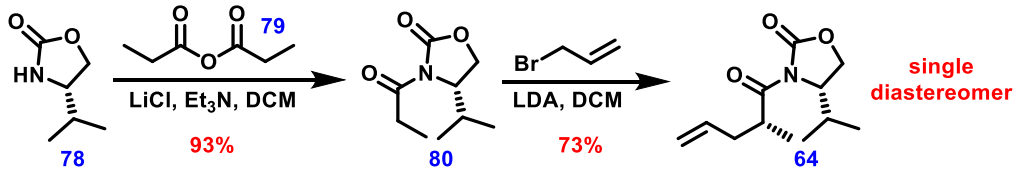
Synthesis of alkene **70** began from commercially available methyl 3-(*S*)-hydroxybutyrate **55** (scheme 43). Following a stereoselective methylation of **55** using LDA and MeI, ester **74** was protected using TBSCl, imidazole and DMAP. Ester **75** was reduced using DIBAL-H to alcohol **76** and Dess Martin periodinane (DMP)[53] used as the oxidant due to the simple purification procedure required.[131] Purification of the crude material by trituration with Et_2O gave the

required product in 96% yield, which was then methylenated under Wittig conditions to give alkene **70**.



Scheme 43. Synthesis of **70** in five steps from **55**.

Alkene **64** was synthesised by reaction of commercially available auxiliary **78** with anhydride **79** in the presence of LiCl and Et_3N to give propionylated oxazolidinone **80** (scheme 44). Allylation of **80** with LDA and allyl bromide gave alkene **64** in 73% yield as a single diastereomer as determined by ^1H NMR spectroscopy.

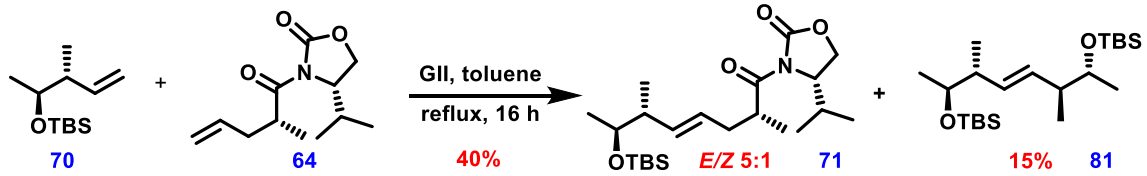


Scheme 44. Synthesis of **64** from **78**.

With both alkenes **70** and **64** in hand, the cross metathesis was investigated. By using the statistical distribution of products model (scheme 26, page 33), four equivalents of alkene **70** and one equivalent of **64** were used as these provided a compromise between material value of the substrates and metathesis selectivity (scheme 45). Grubbs *et al.* reported that with this ratio, up to 80% selectivity for the cross coupled product could be achieved (scheme 26).[98]

Both alkenes **70** and **64** were dissolved in toluene at a concentration of 5 M followed by catalyst addition and the mixture refluxed for 16 h to give the required alkene **71** in 40% yield as a 5:1 mixture of *E*/*Z* isomers (scheme 45). The product of self-metathesis, alkene **81**, was observed by ^1H NMR, however as type I alkene **70** was in excess this side-product was

unsurprising. This side-product **81** was isolated and used as a coupling partner in further cross metatheses, with no detriment to the yield.



Scheme 45. Cross metathesis of **70** and **64**.

As this reaction had only given a 40% yield of the required alkene **71**, a series of different experiments were carried out in order to optimise conditions for this cross metathesis. Careful attention was paid to the concentration of the reaction mixture as the intermolecular reactions needed to be favoured. Most CM reactions are thermoneutral which makes it possible to run reactions neat or very concentrated. In this case, the best yield (53%) was achieved when the reaction was run at a concentration of 10 M.

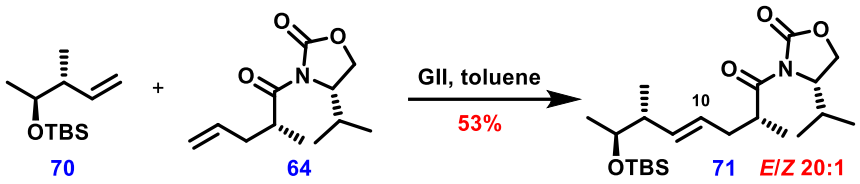
Changes in the order of addition were also explored. It was found that when the alkene in excess (in this case **70**) was premixed with the catalyst and **64** added to the mixture, very low yields of the desired product **71** were obtained. Isolation of the starting materials showed **64** was untouched, however isomerisation of the double bond in **70** had occurred, as well as the product of self-metathesis **81** (scheme 45). The best yields were achieved when alkenes **70** and **64** were premixed and added dropwise to a stirring solution of catalyst in toluene at RT.

Copper iodide has been shown not only to improve the yield of cross metatheses but also to increase the rate, possibly due to the iodide ion acting as a stabilising ligand on ruthenium.[132] Copper (I) acts as a phosphine scavenger which assists in the formation of ruthenium-alkene complexes as the phosphine ligand cannot re-associate with ruthenium in the first step of the catalytic cycle (scheme 25). In addition, Grubbs *et al.* have shown that free phosphine ligands quench the catalyst after a few turnover cycles, so the addition of CuI can increase the longevity of the ruthenium catalyst.[132] However, in the reaction of **70** and **64**, no improvement in yield or rate was observed after the addition of CuI.

The required product alkene **71** was found to coordinate readily with the ruthenium catalyst, which is common in cross metatheses.[133] There are many literature examples of techniques

to circumvent this problem such as adding a water soluble phosphine ligand that coordinates to the ruthenium metal centre allowing the desired product to be isolated cleanly by simple work up procedures, or the addition of lead tetraacetate which binds strongly to the catalyst.[134-136] In this case, flushing the column with neat ethyl acetate after collection of the product containing fractions was found to recover over 10% more product.

By changing the concentration of the reaction and optimising the order of addition of the reagents and catalyst, alkene **71** was isolated in 53% yield with predominantly *E* geometry (scheme 46).



Scheme 46. Cross metathesis of alkenes **70** and **64** following optimisation.

In the ^{1}H NMR spectrum of **71**, the signal assigned to 10-H appeared as a double doublet of triplets (ddt) with coupling constants of 15.5, 8.0 and 1.0 Hz, in accordance with the main product of this CM having *E* geometry (figure 18).

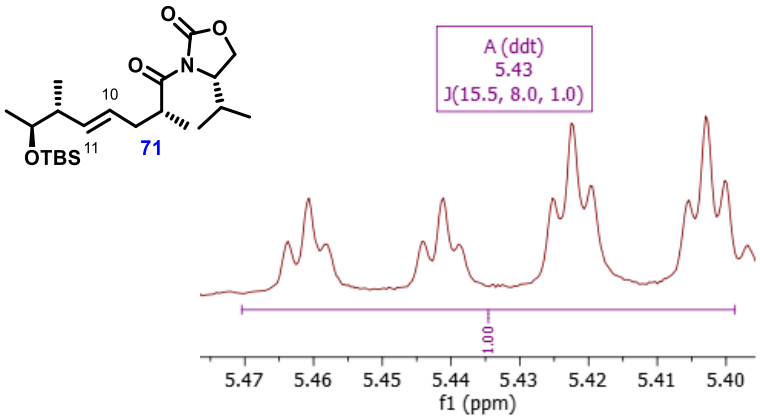
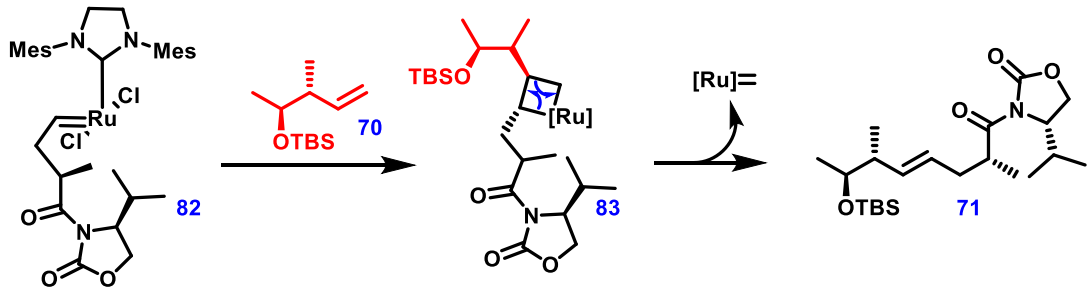


Figure 18. Region of ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) showing signal assigned to 10-H.

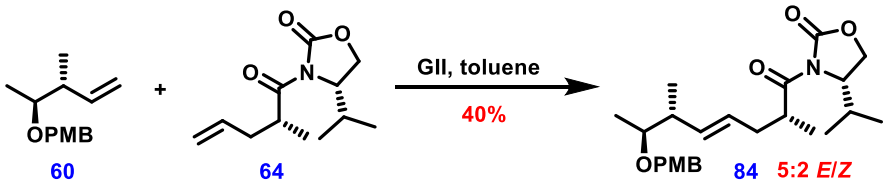
It was envisaged that the ruthenium benzylidene catalyst reacts with the terminal olefin of **64** giving alkylidene **82**. Alkene **70** forms a ruthenacyclobutane **83** in the *anti*-arrangement leading to the more thermodynamically stable *E*-alkene product **71** (scheme 47).



Scheme 47. Proposed geometry of ruthenacyclobutane **83**.

Until relatively recently, there were not many reported methods for ensuring high *trans* selectivity in olefin metatheses. Selectivity can be achieved by long reaction times, ensuring that the thermodynamically favoured product (the *trans* olefin) is present in high concentrations.[137-139] Grubbs and co-workers later published further work on how the stereochemical relationships in the ruthenacyclobutane intermediate can affect the resulting alkene geometry.[140-141] There is experimental evidence supporting olefin binding *trans* to the NHC,[140-141] causing the metallocycle to lie *trans* to the NHC[95] favouring the *trans* olefin product on cycloreversion.

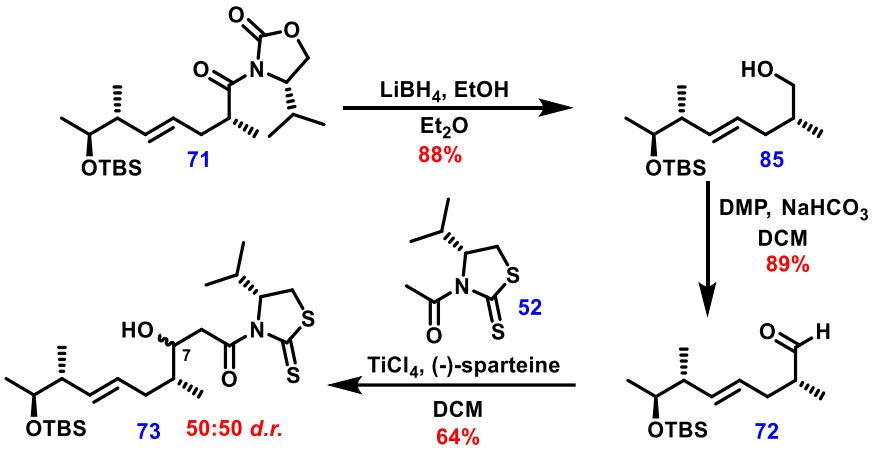
Pleasingly, the reaction of **70** and **64** proceeded with good *E*/*Z* selectivity of approximately 20:1. In comparison to the cross metathesis carried out by Mazzetti (scheme 39), the enhanced *E* selectivity of this reaction could be due to the bulky TBS group providing a steric effect. To investigate the effect of the protecting group, PMB protected alkene **60**, was coupled with alkene **64**, giving **84** in 40% yield and a 5:2 ratio of *E*/*Z* isomers (scheme 48). This reduction in selectivity could be due to the PMB group being less bulky than the corresponding TBS protected olefin **70**, leading to alkene **60** being more able to bind in such a way that the ruthenium metallocycle lies *cis* to the NHC, giving rise to the *cis* (*Z*) product on cycloreversion.



Scheme 48. Cross metathesis of **60** with **64**.

2.3.1 Completing the total synthesis of thioester 28

Following the successful cross metathesis to form **71**, the final steps of the proposed synthetic route (scheme 49) were carried out. The oxazolidinone auxiliary of **71** was reductively cleaved using $LiBH_4$ in EtOH[142] to give alcohol **85** in 88% yield (scheme 49). Oxidation of alcohol **85** by DMP gave aldehyde **72** in 89% yield.



Scheme 49. Cleavage of the auxiliary of **71** to alcohol **85** followed by oxidation and coupling of aldehyde **72** and acylated auxiliary **52**.

An aldol reaction was carried out between aldehyde **72** and acylated thiazolidithione auxiliary **52** using $TiCl_4$ and (-)-sparteine which gave alcohol **73** in 66% yield and as a 1:1 mixture of diastereomers epimeric at C-7. The characteristic signals of the two diastereomers are shown in the ^1H NMR spectrum (figure 19).

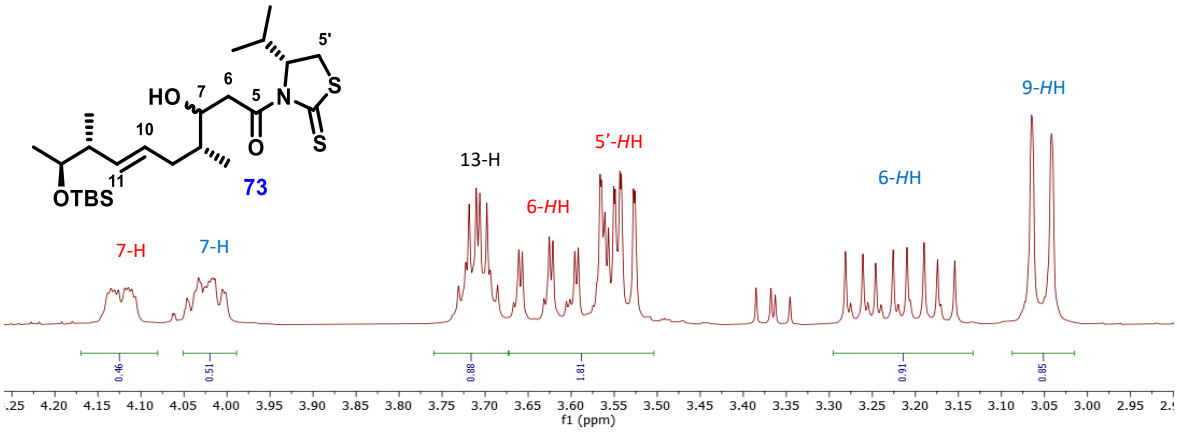


Figure 19. ^1H NMR showing the mixture of diastereomers of **73**. The different coloured labels indicate the different diastereomers to which these protons belong.

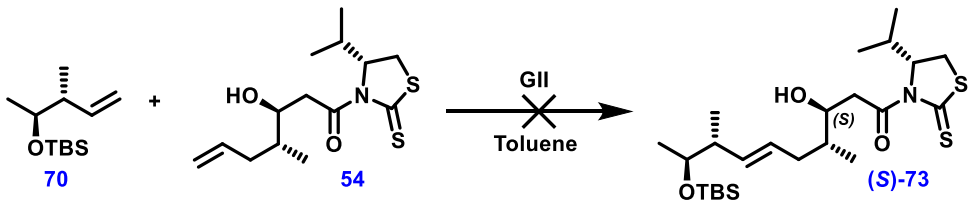
It is interesting to compare the diastereoselectivity of the two aldol reactions as shown in schemes 36 and 49. The lack of selectivity (scheme 49) was hypothesised to be due to the

longer chain on aldehyde **72** compared with aldehyde **30** making the aldehyde too large to be held reliably in the rigid transition state as shown in scheme 36. The aldol reaction between aldehyde **30** and acylated auxiliary **52** as previously discussed (scheme 36) used neat $TiCl_4$, however this reagent has since been discontinued so $TiCl_4$ in DCM was used instead, which could account for the difference in selectivity.

Using the non-chelated Felkin-Ahn model to predict the stereochemical outcome shows that without selectivity and chelation influencing the outcome of this reaction, the major product would have both methyl and hydroxyl groups on the same face, which is another factor that complicates achieving selectivity in this reaction.

Due to the lack of stereoselectivity in this aldol reaction (scheme 49), and as cross metathesis conditions to couple alkenes **70** and **64** had been optimised (scheme 46), it was decided to re-investigate the original cross coupling between alkene **70** and unsaturated alcohol **54** as shown in scheme 50, to determine whether this reaction had failed due to incorrect conditions or an incompatibility of the catalyst and the substrates.

Using the optimised conditions that had been developed in the coupling of alkenes **70** and **64**, (scheme 46), did not give rise to the required product **(*S*)-73** as shown in scheme 50, which suggests this reaction failed due to the incompatibility of the substrates and the catalyst, rather than the conditions (solvent, concentration and temperature).

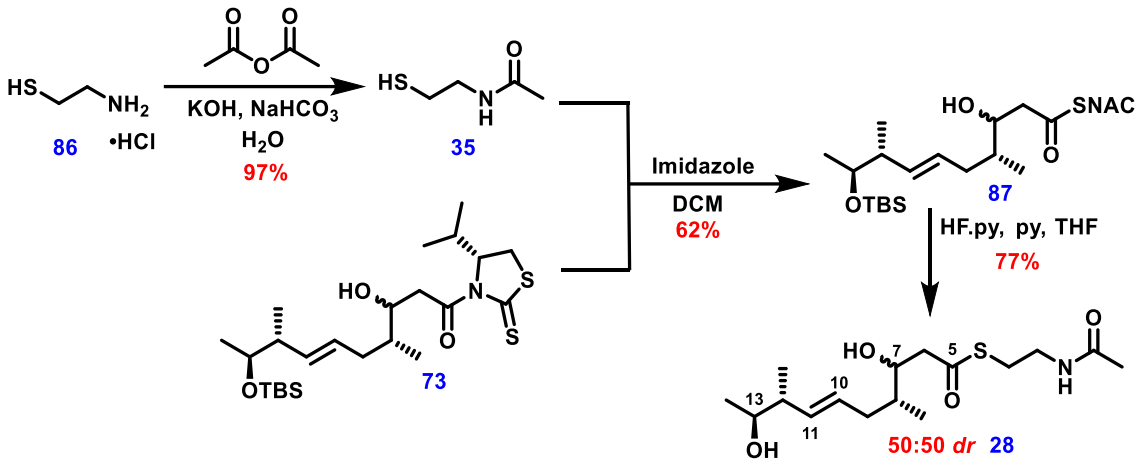


Scheme 50. The attempted cross metathesis of alkenes **70** and **54** using the optimised conditions.

It was decided to continue the synthesis with alcohol **73** as a mixture of diastereomers as it was hoped that the two diastereomers epimeric at C-7 could be separated later in the synthesis by HPLC. Furthermore, both diastereomers would be of interest in exploring the substrate specificities in biotransformations.

It was envisaged that the thiazolidithione **73** could be directly converted to the required substrate **28** for the biotransformations in two steps. First, the required thiol HSNAC **35** was

synthesised in 97% yield from *N*-cysteamine hydrochloride **86** by reaction with Ac_2O, KOH, $NaHCO_3$ following a literature procedure.[143] Treatment of thiazolidithione **73** with imidazole and thiol **35** at room temperature gave the desired thioester **87** in 62% yield after 5 h.[144] The final step required a silyl deprotection, and while there is no literature precedence for the use of TBAF in the presence of an SNAC group, the use of HF.pyridine has been reported.[145] Hence, silyl ether **87** was treated with HF.pyridine at room temperature to give thioester **28** in 77% yield (scheme 51) as a 1:1 mixture of diastereomers epimeric at C-7.



Scheme 51. Final steps to give **28** as a 1:1 mixture of diastereomers.

Analysis of ^{13}C NMR showed the characteristic thioester and amide carbons at 199.9 and 170.7 ppm respectively, the carbons alpha to oxygen: C-13 at 72.4 ppm and two signals for C-7 at 71.5 and 71.3 ppm, and the alkene carbons at 134.4 and 130.2 ppm. As mentioned previously, this substrate was isolated as a 1:1 mixture of diastereomers epimeric at C-7 although this is not immediately obvious as the majority of signals in the ^{13}C NMR overlapped perfectly with the corresponding carbon in the opposite diastereomer, apart from C-7.

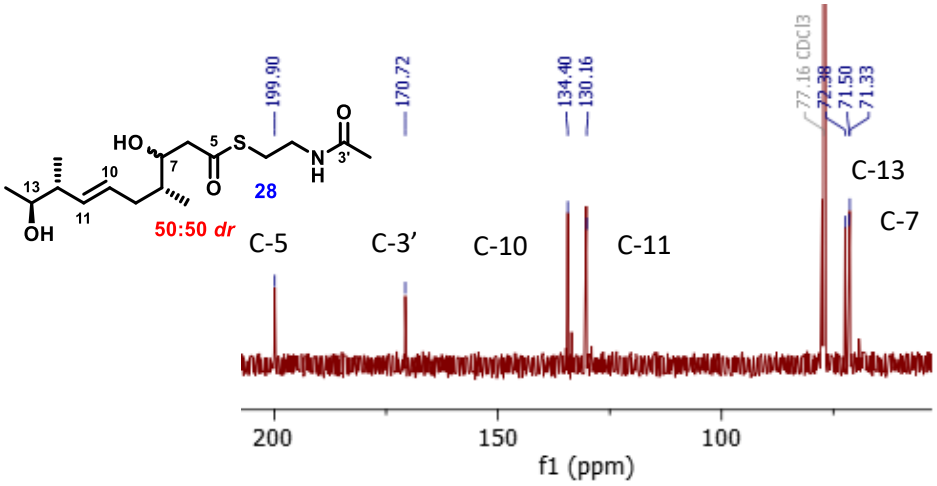


Figure 20. Region of the ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of thioester **28**.

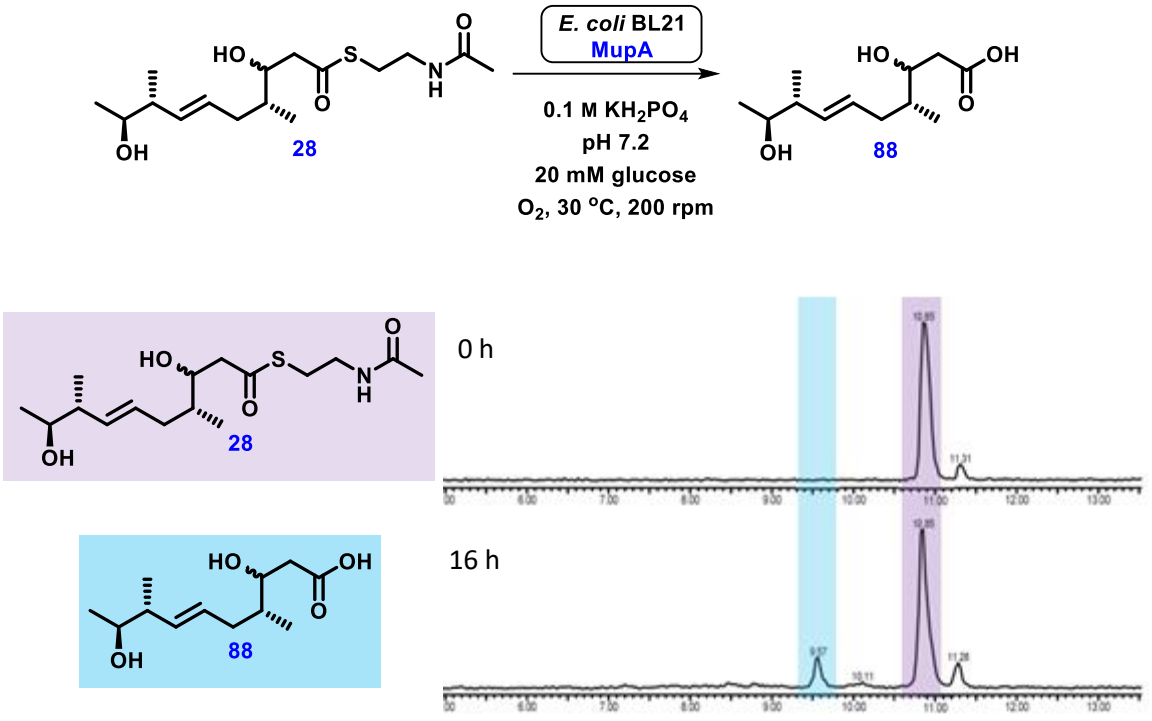
In summary, thioester **28** was synthesised in 11 steps (longest linear sequence) in 4.8% overall yield albeit as a mixture of diastereomers epimeric at C-7. Although separation of these diastereomers was attempted by both chiral HPLC and standard reverse phase HPLC conditions, the retention times proved too similar to achieve separation. It was decided to carry out the biotransformation studies with the hope that the diastereomer with the 'natural' stereochemistry at C-7 would be recognised and turned over by MupA.

2.4 Biotransformation studies of thioester 28 with MupA

With thioester **28** in hand, investigations into the putative function of MupA as the 6-hydroxylase could be carried out using *in vivo* biotransformations in *E. coli*. Two identical feeding studies and a negative control experiment (denatured enzyme boiled at 95 °C for 10 mins) were carried out. The biotransformation mixtures (1 mL) contained 0.2 g *E. coli* cells which had been modified to contain a plasmid for MupA expression by Dr Luoyi Wang. Each reaction contained 100 mM potassium phosphate buffer pH 7.3, 20 mM glucose and thioester **28** dissolved in MeOH (100 µL) as shown in scheme 52. These were incubated at 30 °C and shaken at 200 rpm for 24 h. After this time, the reactions were quenched by the addition of acetonitrile, vortexed and centrifuged. The organic layer was analysed by HPLC-MS.

Whole cells were used in these initial experiments as it was thought they would contain the relevant co-factors that MupA requires to carry out the biotransformation. After 24 h, HR-MS

revealed that the ester had been hydrolysed to give carboxylic acid **88** as shown in scheme 52, and repeated experiments gave the same result.



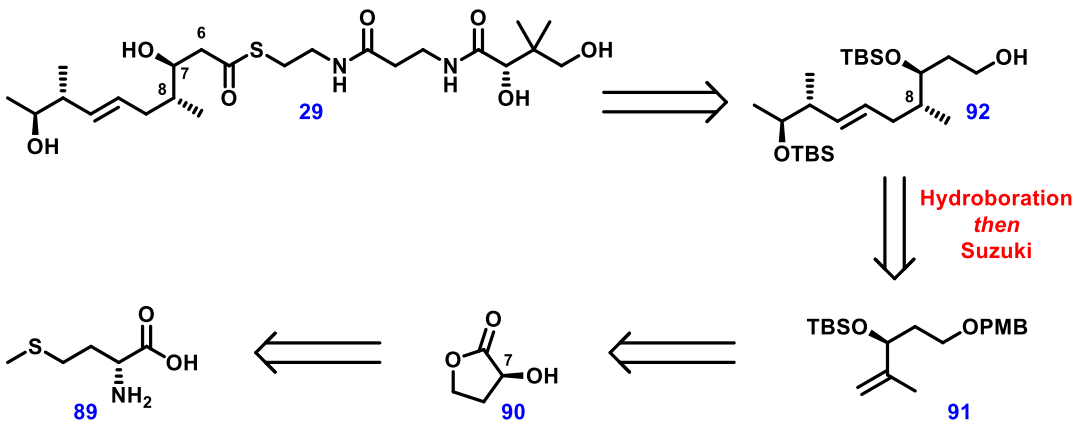
Scheme 52. HPLC trace showing hydrolysis of thioester **28** to acid **88**.

These results showed that substrate **28** was not recognised by the system possibly due to the SNAC side chain not being similar enough to the native ACP and so with this in mind, work was started on the synthesis of **29** bearing the pantetheine side chain.

2.5 Synthetic efforts towards substrate 29

Whilst feeding studies with SNAC derivatives have been successful in investigations of polyketide biosynthesis,[146] greater success may be achieved using the longer pantetheine side-chain, thus, the next goal was to prepare thioester **29**.

A new approach to the synthesis of thioester **29** was devised due to the difficulties experienced in achieving stereoselectivity at C-7 in the synthesis of **28**.

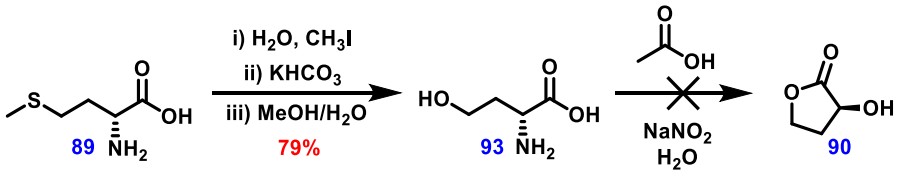


Scheme 53. The proposed synthesis of **29** from **89**.

This new route involved two key disconnections, a one-pot hydroboration followed by Suzuki cross coupling, which introduced the stereocentre at C-8, and a thioesterification to install the pantetheine side-chain. This synthesis used key elements Zhao *et al.* described in the total synthesis of mupirocin H (scheme 22, page 28).

2.5.1 Synthesis of protected unsaturated diol 99

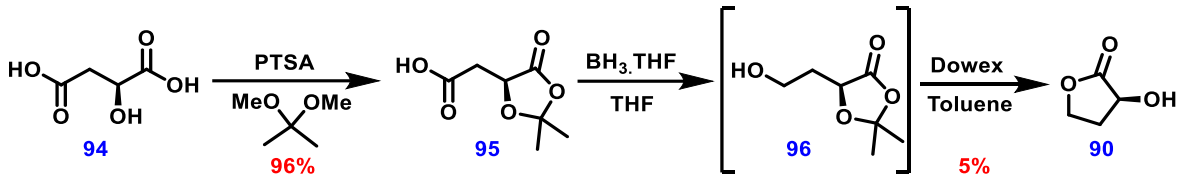
This new approach started by converting commercially available D-methionine **89** into D-homoserine **93** using H_2O and MeI followed by $KHCO_3$ and $MeOH/H_2O$ (scheme 54). Treatment of D-homoserine **93** with acetic acid followed by $NaNO_2$ forms a metastable diazonium intermediate, which on addition of water, releases nitrogen gas and dihydroxybutanoic acid is formed. Although this is a literature reaction, in my hands hydroxy lactone **90** was not observed by ^1H NMR spectoscopy.[147]



Scheme 54. Synthesis of hydroxy lactone **90** from D-methionine **89**.

Another approach to hydroxy lactone **90** started from commercially available L-malic acid **94** which was protected to give acid **95** (scheme 54).[147] Selective reduction of acid **95** with BH_3.THF gave alcohol **96**, which was treated with PTSA *in situ*. It was reported acetonide deprotection and spontaneous cyclisation should occur,[148] however in my hands none of the

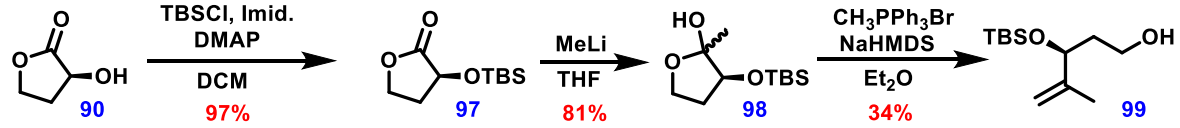
required hydroxy lactone **90** was isolated. When alcohol **96** was treated with Dowex the product **90** was isolated in 5% yield.



Scheme 55. The synthesis of hydroxy lactone **90** from L-malic acid **94**.

Monitoring the reaction by both ^{1}H NMR and IR showed that reduction of acid **95** to alcohol **96** was taking place, however after the isolation of alcohol **96**, further analysis showed that the acetonide protecting group had been lost prior to the addition of the acid catalyst.

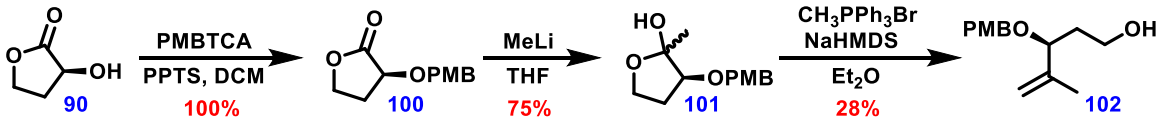
Hydroxy lactone **90** is commercially available, so it was purchased, and the synthesis continued by protection of the hydroxyl group as the TBS ether using standard protection conditions (scheme 56). Addition of MeLi to hydroxy lactone **97** gave lactol **98** in 81% yield. Wittig conditions were used in the subsequent methylenation, however only a modest yield (34%) of unsaturated alcohol **99** was obtained.



Scheme 56. Synthesis of alkene **99** from hydroxy lactone **90**.

Analysis of lactol **98** by ^{1}H NMR showed that it existed exclusively as the lactol tautomer, and not as the ring opened methyl ketone which is the required substrate for methylenation. This could explain why no consumption of starting material was observed when the reaction was carried out at RT, as heating to reflux for 24 hours was required for this ring opening to take place and therefore methylenation to occur.

Wittig reactions are also known to be sensitive to steric bulk surrounding the carbonyl group undergoing the reaction [149-150] and so to investigate this hydroxy lactone **90** was protected with the less bulky PMB protecting group using PMBTCA to give PMB ether **100** as shown in scheme 57. Addition of MeLi gave protected lactol **101**, which was methylenated under Wittig conditions to give alkene **102** in 28% yield.

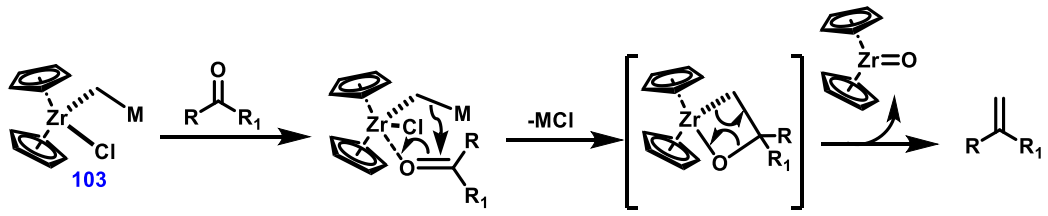


Scheme 57. Synthesis of alkene **102**.

The basic character of the ylide in the Wittig reaction is known to be incompatible with some sensitive carbonyl-containing compounds, especially readily enolisable ketones. Many of the classic, widely used Wittig reagents can act to remove the acidic proton α to the carbonyl group making proton abstraction the dominant reaction.[138]

Due to the low yields achieved using Wittig conditions (scheme 56 and 57) it was decided to explore other methylenation conditions. Methylenations carried out using organotitanium or organozirconium reagents have proven particularly useful for transformations on hindered or base sensitive carbonyls.[151] The basicities of these species are comparatively weaker than Wittig salts, which enables easily enolisable substrates to be used and gives the desired olefins in high yields and short reaction times, while also reducing the risk of epimerisation taking place at enolisable asymmetric centres.[152]

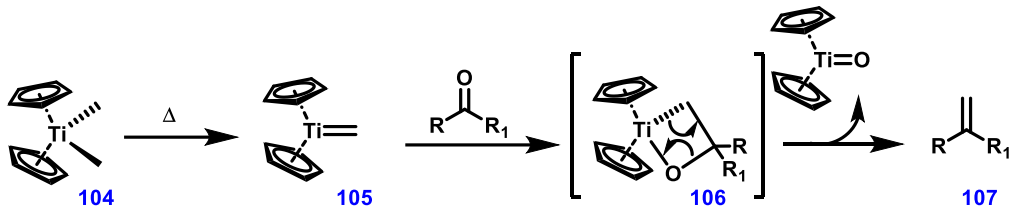
In 1989 Tour reported the use of a zirconium complex as a methylenating agent. Treatment of zirconocene dichloride with dibromomethane and zinc affords an organometallic intermediate **103** (scheme 58) which rapidly methylenates aldehydes, ketones, and enones at room temperature.[152] It is seen as a milder alternative to organotitanium reagents as zirconium is less Lewis acidic than titanium.[152] Treatment of lactol **76** with the conditions specified by Tour *et al.* gave *ca.* 1% yield of the desired product.[152]



Scheme 58. The proposed mechanism for methylenation under Tour's conditions.[152]

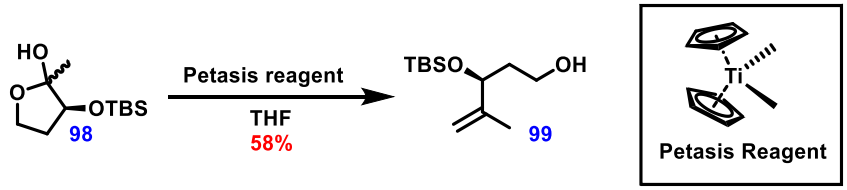
Petasis reagent **104** (scheme 59) has been reported to be an effective methylenating agent for hindered carbonyls, with the active species being Schrock carbene **105** (scheme 59).[153] Once this carbene **105** has been formed, it reacts with the oxygen of carbonyl compound to give a hypothesised oxatitanacyclobutane intermediate **106**. In the final step of the

mechanism, this oxatitanacyclobutane **106** breaks down to give the desired olefin **107**, the driving force of which is the high oxophilicity of Ti(IV).[151]



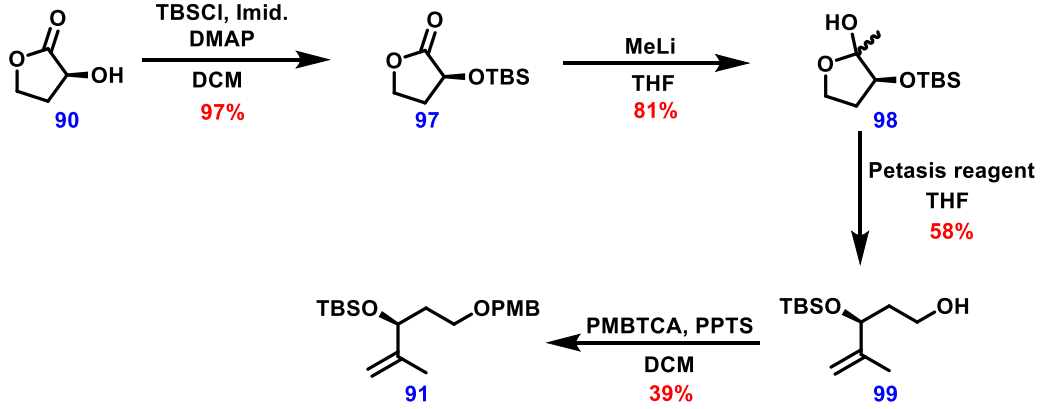
Scheme 59. The proposed mechanism of methylenation using Petasis reagent.[151]

Refluxing protected lactol **98** in a solution of Petasis reagent in toluene for 16 h gave **99** in 48% yield (scheme 60), however a difficult acidic work-up procedure precluded this reaction being carried out on a large scale. Addition of ethanol to the reaction mixture after full consumption of starting material, and heating for 6 hours at 60 °C followed by a simple filtration proved effective in removing any titanium by-products,[154] and enabled this reaction to be scaled up effectively, giving the required alkene in 58% yield.



Scheme 60. Optimised methylenation of lactol **98** to give alkene **99**.

Alcohol **99** was protected as a PMB ether **91** using PMBTCA and PPTS as shown in scheme 61, as this was orthogonal to the other protecting group strategies used in the synthesis of thioester **29**.

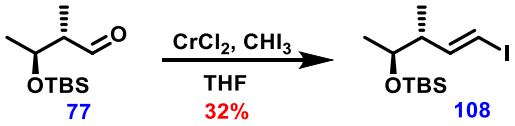


Scheme 61. Synthesis of alkene **91**.

To summarise, core fragment **91** was synthesised in four steps from the commercially available hydroxy lactone **90** in 18% overall yield (scheme 61). Initially, Wittig conditions were used in the methylenation of lactol **98**, however modest yields (34%) were obtained even when a less bulky protecting group was used. After subjecting protected lactol **98** to a number of different methylenation conditions, the best yield (58%) of alkene **99** was achieved when using Petasis reagent.

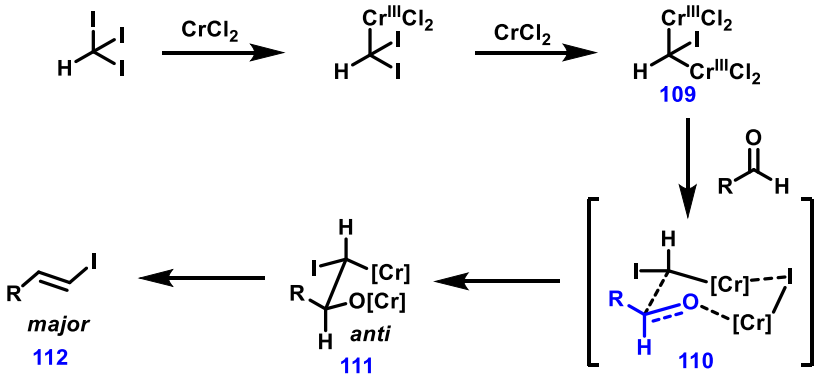
2.5.1 Synthesis of *trans* vinyl iodide 108

With an effective synthetic route to alkene **91** established, the next steps in this synthetic route involved the synthesis of *trans* vinyl iodide **108** required for the key Suzuki cross coupling. Using the synthetic route established to terminal alkene **70** in the synthesis of thioester **28** (scheme 43, page 42), modifications were made in order to synthesise *trans* vinyl iodide **108** from aldehyde **77** using Takai's conditions as shown in scheme 62.[155]



Scheme 62. Synthesis of *trans* vinyl iodide **108** from aldehyde **77**.

Transformation of aldehyde **77** to *trans* vinyl iodide **108** utilised Takai's methodology which is highly selective for the formation of (*E*)-alkenes.[155] Hodgson *et al*. and Takai *et al*. have proposed very similar mechanisms which explain the (*E*)-stereoselectivity observed in this reaction (scheme 63).[156-157] It is proposed that iodoform is converted to a nucleophilic geminal dichromium complex **109** by reaction with $CrCl_2$.



Scheme 63. The proposed intermediates in the formation of *trans* vinyl iodides.

The oxygen of the aldehyde coordinates to the chromium centre forming a six-membered pseudo-chair transition state **110** containing two chromium atoms bridged by iodine as shown in scheme 63. The aldehyde side-chain and the iodide occupy the sterically favoured equatorial positions, such that the chromium adduct **111** exists in the *anti*-conformation. It is proposed that C-C bond rotation takes place at the same time as *syn* elimination to give the *trans* vinyl iodide **112** as the major product. Alkenyl halides with *trans* geometry are produced almost exclusively when iodoform is used, the ratio decreasing with bromoform and more so with chloroform.[155] The reaction of aldehyde **77** with CHI_3 and $CrCl_2$ proved to be very sensitive to concentration, temperature and scale. The best yield (49%) was achieved when the reaction was run at 0 °C and 1 M concentration. Pleasingly, total control of the geometry was achieved (figure 21) and ^{1}H NMR analysis of the 11-H proton (PA numbering) showed the coupling constant of this proton to the 10-H proton to be 14.5 Hz, which is in accord with a t*rans* relationship.

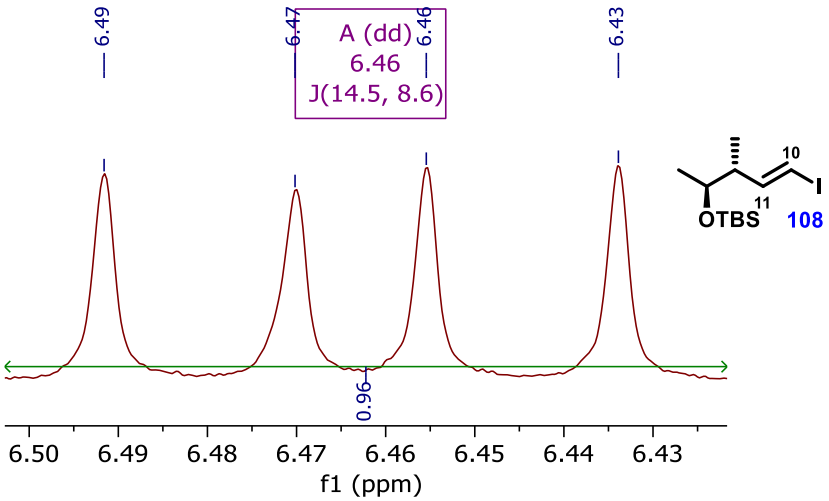
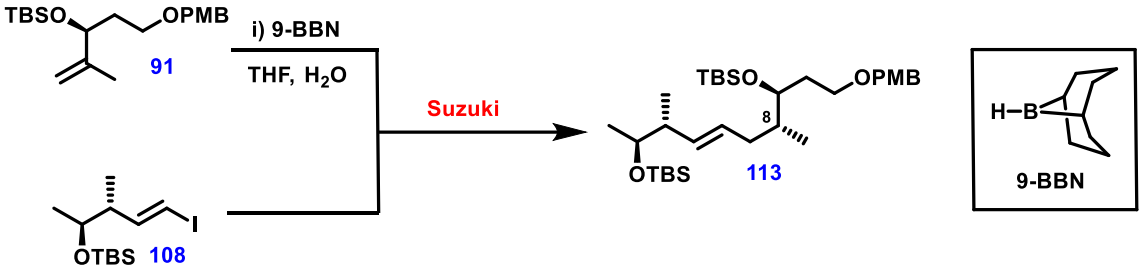


Figure 21. An excerpt from the ^{1}H NMR spectrum of **108**, showing the *trans* relationship between 11-H and 10-H.

2.5.2 Hydroboration of alkene 91 and Suzuki reaction with vinyl iodide 108

With *trans* vinyl iodide **108** in hand, the hydroboration of alkene **91** was undertaken prior to the Suzuki cross coupling. Terminal alkene **91** was stirred for 16 hours with the hindered organoborane 9-borabicyclo[3.3.1]nonane (9-BBN), followed by the addition of water, which was degassed in order to eliminate any risk of catalyst poisoning in the subsequent Suzuki

reaction. The resulting organoborane was not isolated and was used *in situ* with *trans* vinyl iodide **108** (scheme 64).



Scheme 64. The hydroboration of **91** and subsequent Suzuki coupling with **108**.

Hydroborations usually proceed to give the *anti*-Markovnikov product with *syn* stereochemistry.[158] The borane is added to the least hindered carbon, allowing the partial positive charge to form on the more substituted carbon as proposed by the *anti*-Markovnikov rule. Houk *et al*. showed from computational studies using the 3-21G basis set that a methyl substituent at the alkene terminus away from boron lowers the activation energy of this transition state which is consistent with the idea that this is an electrophilic process, and further confirms the hypothesis that the borane attacks at the least hindered carbon.[159]

Calculations by Houk *et al.* assessed the steric requirements of each group attached to a central carbon. They found that the largest groups had to be antiperiplanar to the forming bond which is clearly the least sterically hindered position. The next largest group should be away from the double bond "outside" the transition state, with the smallest group "inside" the transition state, however this does not take into account electronic effects.[159]

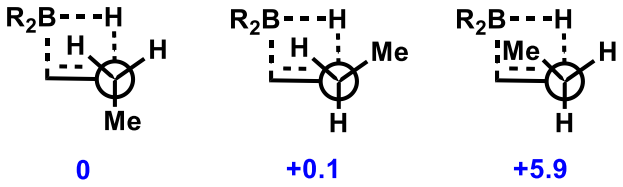


Figure 22. The relative energies (kcal/mol) of the different transition state models.[159]

Houk *et al.* went on to investigate the electronic effect of an allylic alcohol on the proposed transition state.[159] The transition state with the lowest activation energy showed the alcohol "inside" the transition state, the proposed reason for this being that when the allylic CO bond is nearly in the plane of the alkene, it withdraws less electron density from the bond

(compared to if it was perpendicular). If it were perpendicular, then the overlap of the σ* with the alkene π orbital would destabilise the electrophilic transition state.

Evans *et al.* explored the difference between electronic effects and steric effects on the different transition states. He noted that when a small boron reagent was used, transition state **S1** was much more favoured than **S2** and so the opposite facial selectivity of hydroboration is achieved than when using a bulky boron reagent (figure 23).[160]

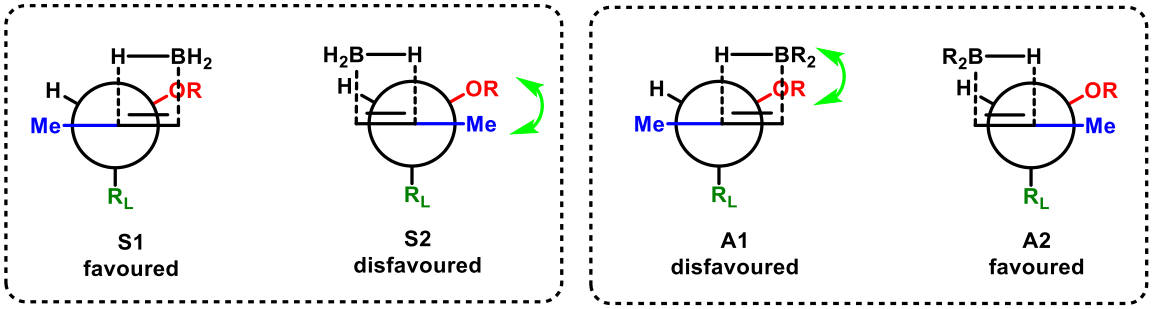
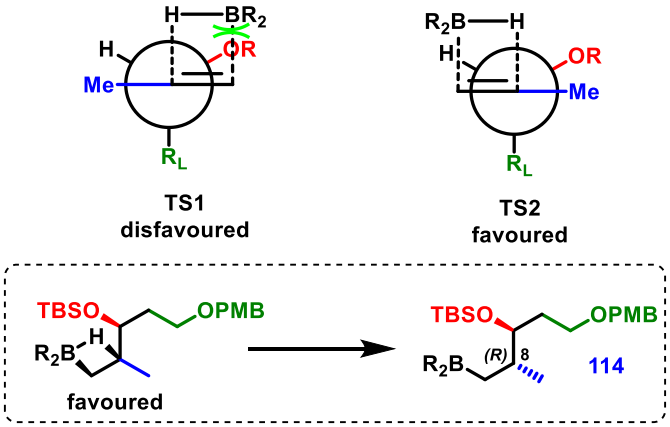


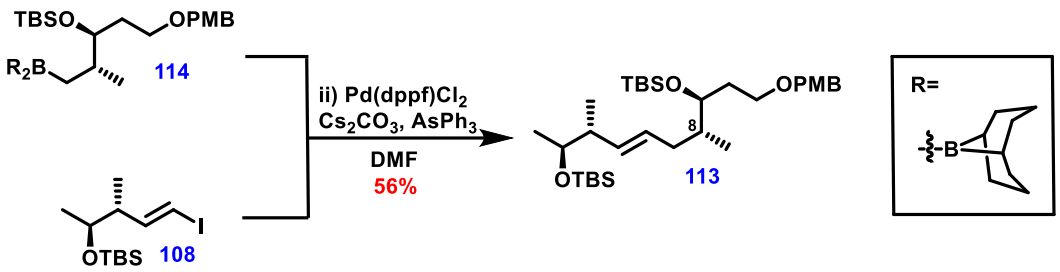
Figure 23. The difference in facial selectivity when a small (left) and a large (right) boron reagent is used.[160]

In the case of alkene **91**, the allylic alcohol is protected with a large silyl protecting group and a bulky organoboron reagent is used, so it is assumed that steric effects play a bigger role in the transition state formation than electronic effects. All of these factors rationalise the formation of a single diastereomer at C-8 (scheme 65).



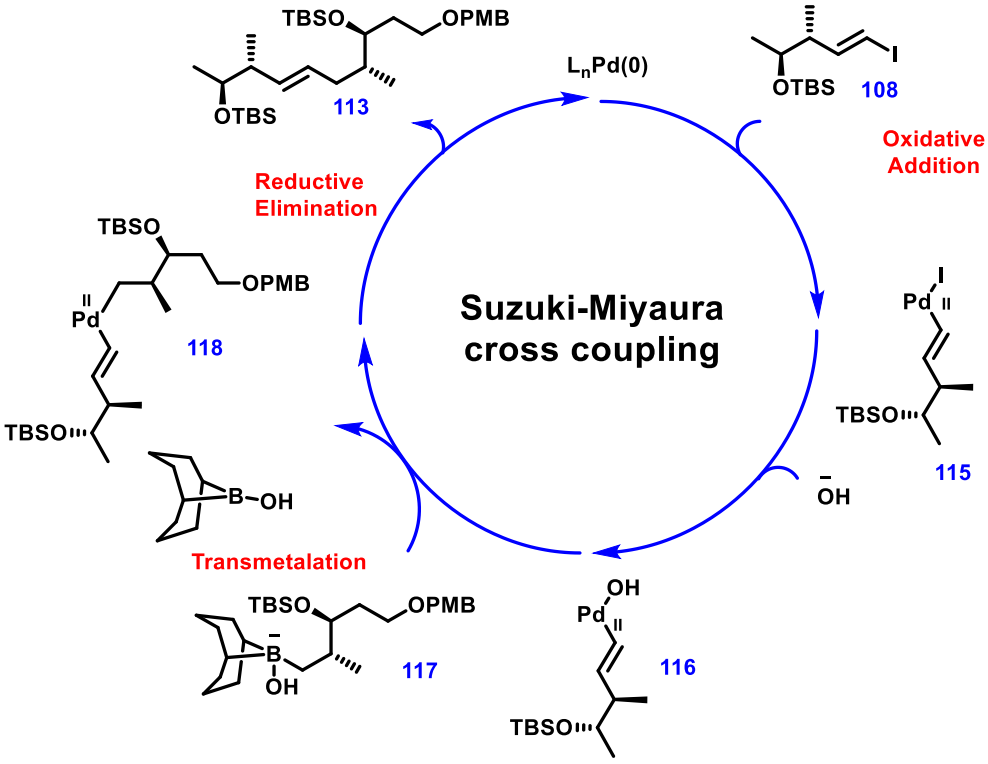
Scheme 65. The proposed transition states in hydroboration of alkene **91** to give organoborane **114**.

With both *trans* vinyl iodide **108** and organoborane **114** synthesised, the Suzuki coupling was carried out using $Pd(ddpf)Cl_2$ as the catalyst, to give alkene **113** with *E* geometry as a single diastereomer in 56% yield over two steps.



Scheme 66. The Suzuki reaction between *trans* vinyl iodide **108** and organoborane **114**.

Since the advent of cross coupling chemistry, the Suzuki reaction has been a popular way of bringing together complex units. The proposed catalytic cycle proceeds as shown in scheme 67. Oxidative addition of the vinyl iodide to the Pd(0) complex in the first step yields a stable trans-palladium (II) complex **115**.[161]

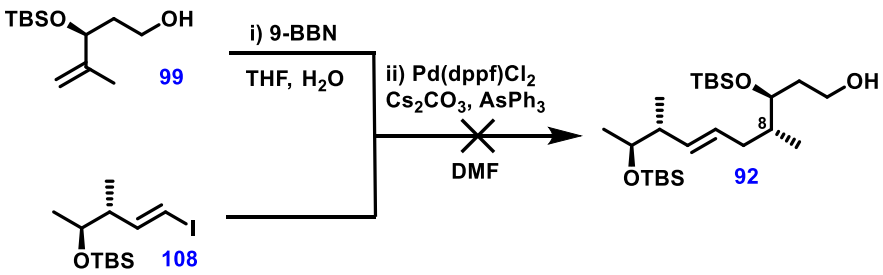


Scheme 67. The catalytic cycle of the Suzuki cross coupling.

Transmetallation takes place in the next step of the catalytic cycle in the presence of a suitable base and ligand, in this case Cs_2CO_3 and $AsPh_3$, producing a Pd (II) complex. Halogen exchange to the more reactive organopalladium hydroxide **116** accelerates this step. In the final step, reductive elimination gives **113** and regenerates the Pd(0) catalyst.[161]

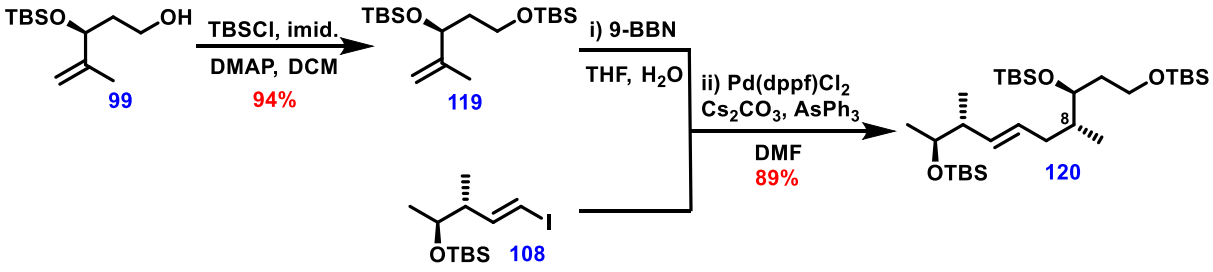
There are a wide range of air stable palladium catalysts that can be used in the Suzuki reaction such as $Pd(OAc)_2$ and $Pd(PPh_3)_4$ which can be readily reduced to Pd(0). In this case $Pd(dppf)_2Cl_2$ was used as it had been used successfully in similar reactions within in the group.[162]

In order to determine whether the protecting group on the primary alcohol of organoborane **114** was influencing both the efficacy of the coupling and the stereochemistry of the methyl group at C-8, model studies were carried out using *trans* vinyl iodide **108** and alkenes **99** and **119** (schemes 68 and 69). When unsaturated alcohol **99** was hydroborated using 9-BBN, and the resulting organoborane reacted with *trans* vinyl iodide **108** under the conditions shown in scheme 68, no reaction was observed, and vinyl iodide **108** and the organoborane were isolated by column chromatography. Limited studies carried out in this area have proposed that hydroxyl groups can form hydrogen bonds with Pd(II), reducing them to Pd(0) and therefore rendering the catalyst inactive.[163]



Scheme 68. The unsuccessful coupling of **99** and **108**.

Alkene **119** was synthesised in one step from core fragment **99** in 94% yield (scheme 69). When bis-TBS protected alkene **119** and *trans* vinyl iodide **108** were subjected to the same conditions as shown in scheme 66, the reaction proceeded with 89% yield and the product **120** was isolated as single diastereomer at C-8 with the *E*-alkene (*J* 15.0 Hz) as determined by ^{1}H NMR.

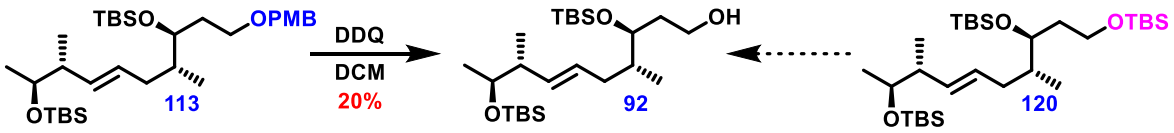


Scheme 69. Synthesis of **120**.

The product **120** was recrystallised from petrol and although some single crystals were produced, the needle-like crystals were deemed to be too thin to diffract the light and so were unsuitable for X-ray crystallography to confirm the stereochemistry of the methyl group. It was decided to continue with the synthesis and compare the data of the final product to data from isolated natural products, which would provide confirmation of the stereochemistry at this position.

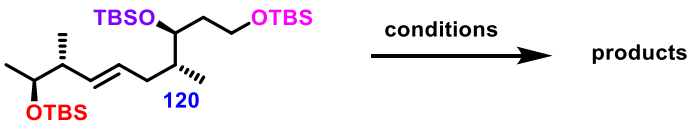
2.5.3 Completion of the total synthesis of 29

With both protected triols **113** and **120** in hand, the next challenge was to selectively remove the protecting groups on the primary alcohols to generate **92** (scheme 70).



Scheme 70. The two deprotection strategies to give **92**.

First, PMB ether **113** was stirred with DDQ for 5 h at RT to give the required alcohol **92** in 20% yield. As this was a relatively low yield, conditions were investigated to selectively remove the primary silyl ether of **120** in the presence of the secondary silyl ethers.[164]



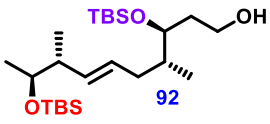
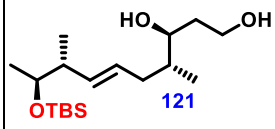
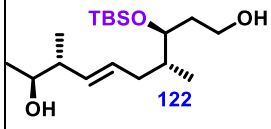
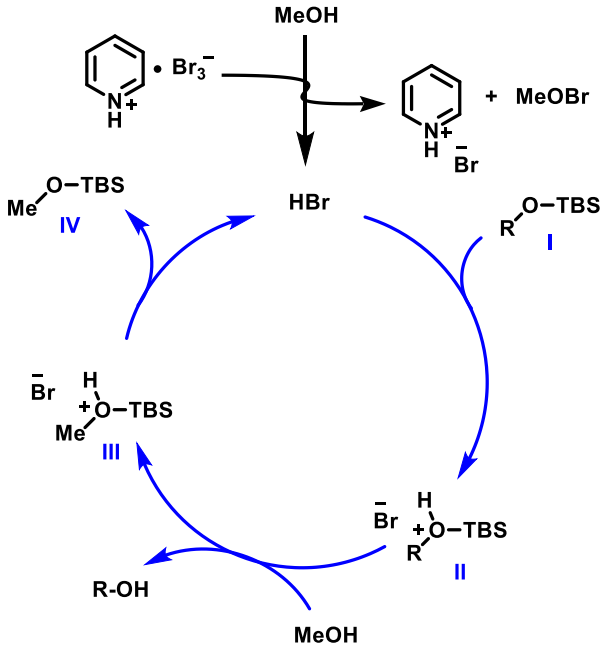
Conditions \ Products			
10 eq. TBAF, THF, RT 24 h	-	74%	-
0.05 eq. $Py.Br_3$, MeOH 0 °C, 3 h	40%	-	-
0.1 eq. $PyBr_3$, MeOH 0 °C, 6 h	10%	-	75%
0.005 eq. $NaAuCl_4.H_2O$ MeOH, 0 °C, 6 h	25%	-	25%

Table 3. Different conditions used in the selective deprotection of **120**.

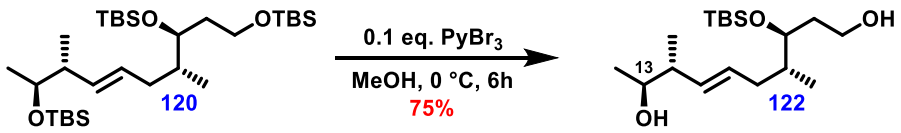
The best yield of required alcohol **92** was achieved when 0.05 equivalents of $Py.Br_3$ were used. Jennings *et al.* first used these conditions when trying to recreate the work of Patel *et al.* who noted that when tetrabutylammonium tribromide (TBATB) was used in silyl deprotections, a primary TBS ether could be removed in a matter of minutes as opposed to hours for a secondary TBS ether.[165]

The working hypothesis for this reaction suggests that pyridinium tribromide goes through the same catalytic cycle as TBATB (scheme 71). Catalytic amounts of HBr are formed in the first step, which protonates the TBS ether forming oxonium cation **II** which undergoes nucleophilic displacement by MeOH to give cation **III**, releasing the desired primary alcohol. In the final step the acid catalyst is reformed so the cycle can continue as shown in scheme 71.[165]



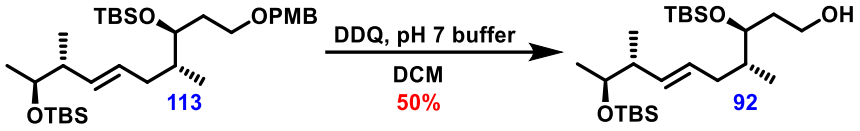
Scheme 71. The proposed catalytic cycle generating HBr used in silyl deprotections.[165]

Although Jennings *et al.* specifies these conditions will selectively remove a primary TBS ether in the presence of a secondary TBS ether,[165] achieving reproducible results in the deprotection of protected triol **120** proved challenging. When 0.1 equivalents of the catalyst were used under literature conditions as shown in scheme 72, monoprotected triol **122** was isolated as the major product (75% yield). Using 0.05 equivalents of the catalyst gave exclusively the desired product in 40% yield, however this proved to be irreproducible.



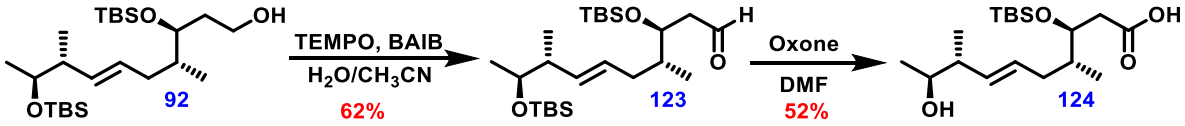
Scheme 72. Deprotection of TBS ether **120** to monoprotected alcohol **122**.

As it was proving difficult to achieve selectivity for the mono-deprotected alcohol **92** from silyl ether **120**, optimisation of the deprotection of PMB ether **113** using DDQ was carried out as shown in scheme 73. It was found that when by adding 5% pH 7 buffer to the solvent mixture, the yield of alcohol **92** increased to 50%.[166]



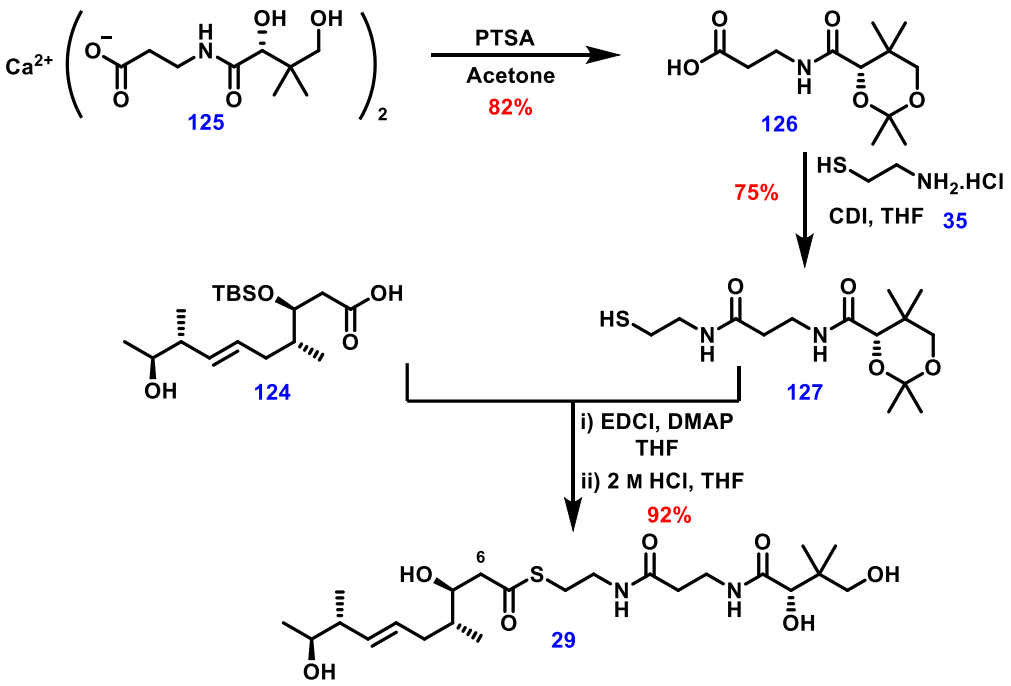
Scheme 73. The optimised deprotection of PMB ether **113** to alcohol **92**.

To complete the total synthesis of **29**, oxidation of alcohol **92** with TEMPO/BAIB in MeCN/H_2O was carried out which gave aldehyde **123** in 62% yield (scheme 74). Aldehyde **123** was then oxidised with Oxone which not only converted aldehyde **123** to a carboxylic acid, but also removed one of the silyl protecting groups to give **124**, which was not surprising as Oxone is a known silyl deprotecting agent.[167]



Scheme 74. Oxidation of alcohol **92** to acid **124** via aldehyde **123**.

Protected pantetheine **127** was synthesised in two steps by first treating commercially available calcium D-pantothenate **125** with PTSA in acetone to give protected pantothenic acid **126**, which was coupled to *N*-cysteamine hydrochloride **35** in the presence of carbonyldiimidazole (CDI) to give the desired product **127** in 75% yield (scheme 75). Coupling of acid **124** with protected pantetheine **127** in the presence of EDCI/DMAP proceeded cleanly, and following silyl deprotection, gave the required product **29** in 92% yield.

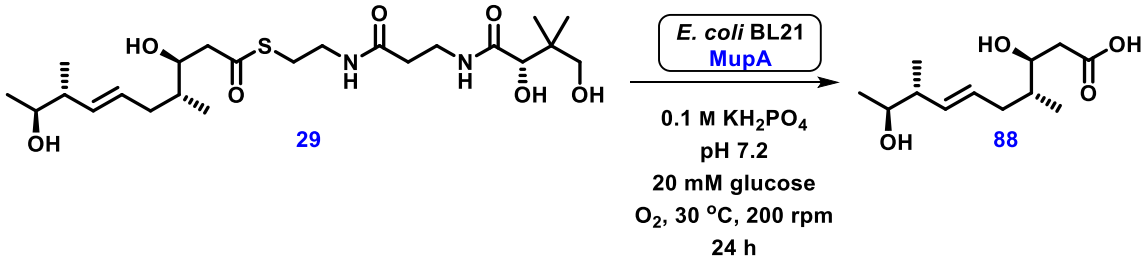


Scheme 75. Coupling and final deprotection to give thioester **29**.

The second substrate for biotransformation studies, thioester **29**, was synthesised in 12 longest linear steps and 3.3% overall yield. Pleasingly, all four stereocentres were set with total stereocontrol.

2.6 Biotransformation studies of pantetheine linked substrate 29 with MupA

Biotransformation studies with substrate **29** and MupA were carried out using the same methodology as discussed in section 2.4 (page 49). Substrate **29** was incubated with *E. coli* whole cells expressing MupA at 30 °C and shaken at 200 rpm for 24 hours. After this time, the reaction was quenched by addition of acetonitrile, vortexed and centrifuged. LCMS-MS analysis of the organic extract revealed that thioester hydrolysis had occurred to produce acid **66** (scheme 76).



Scheme 76. Hydrolysis of thioester **29** to give acid **88**.

2.6.1 Upgrade of substrate 29 onto mmpD-ACP-4

As both substrates, thioester **28** and pantetheine **29**, had not been turned over by MupA, it was decided to carry out work to install the full ACP side chain onto thioester **28**. It was hypothesised that this transformation takes place on the final ACP in *mmpD* (mmpD-ACP-4) as shown in figure 24 and therefore thioester **29** was upgraded onto this ACP as shown in scheme 77. This work was undertaken by Dr Ash Winter of the Crump group in Bristol.

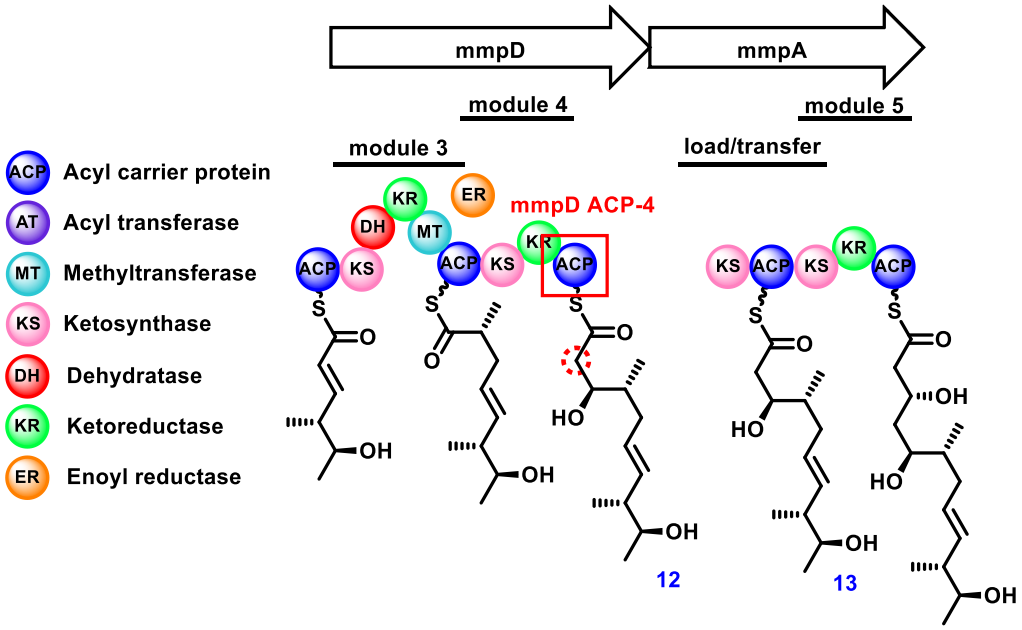
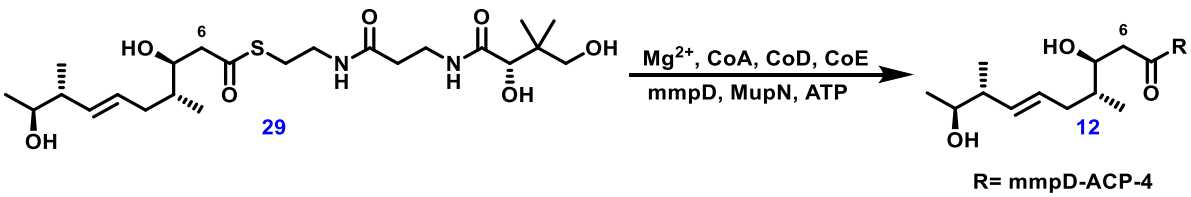


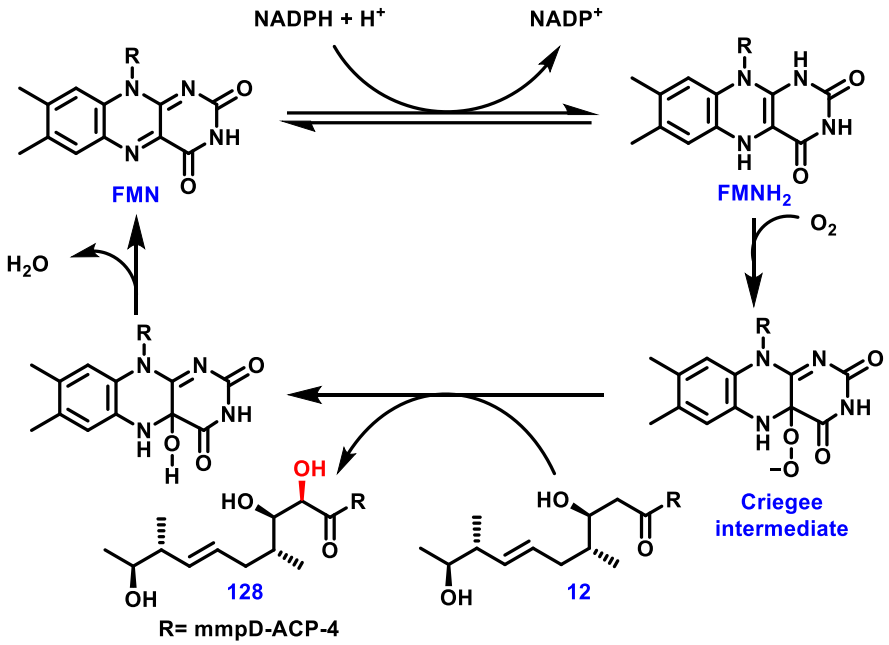
Figure 24. The final ACP in *mmpD* and the proposed site of 6-hydroxylation (red circle).

This process was carried out by adding an excess of thioester **29** to the desired ACP (mmpD-ACP-4). Magnesium was added in the form of hydrated $MgSO_4$ as a stock solution in water as this is required by MupN – a phosphopantetheinyl transferase. Co-enzymes A, D and E were added, which upgraded the substrate onto CoA. This chain extended substrate was then loaded onto the ACP by MupN using ATP (scheme 77).



Scheme 77. Upgrade of substrate **29** onto mmpD-ACP-4.

The mechanism of the 6-hydroxylation, catalysed by MupA, is proposed to proceed as shown in scheme 78. Reduced flavin $FMNH_2$, reacts with molecular oxygen to generate the stable flavin-peroxide intermediate (Criegee intermediate). Following hydroxylation of the substrate, a hydroxy-flavin adduct is produced which is dehydrated to regenerate FMN.



Scheme 78. The proposed mechanism for 6-hydroxylation of substrate **104**.

When substrate **12** was incubated with MupA, $FMNH_2$ and NADPH in the presence of O_2, none of the expected 6-hydroxylated material **128** was observed. An NMR assay, carried out by Dr Winter, showed no apparent interaction of MupA with mmpD-ACP-4, indicating that MupA was not acting on that ACP, as the interaction between the two proteins (MupA and mmpD-ACP-4) should be visible by NMR (figure 25).

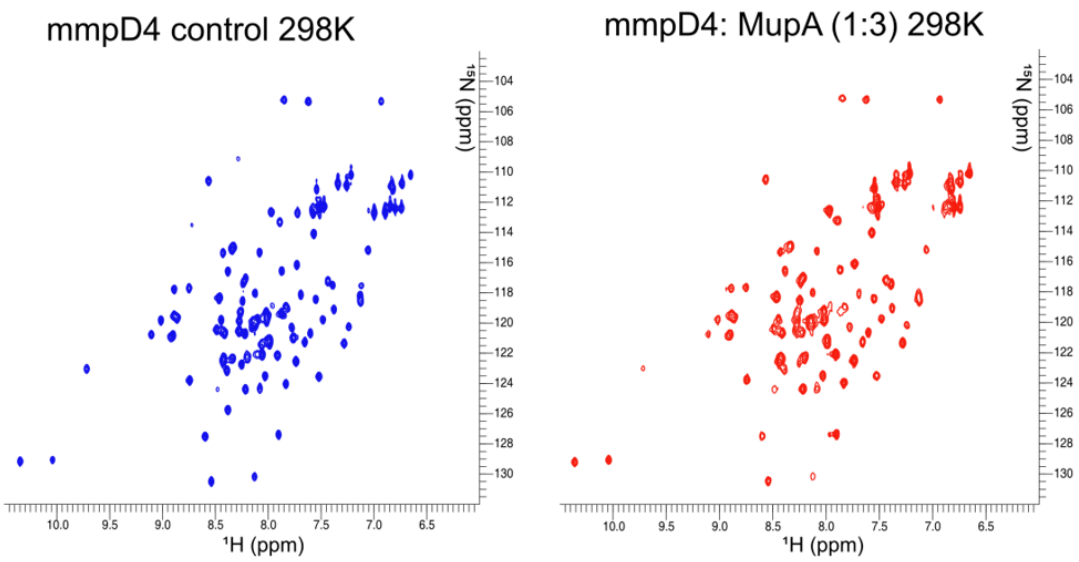


Figure 25. NMR assays of mmpD-ACP-4 (left) and after the addition of MupA (right).

This type of NMR assay is designed to show protein-protein interactions. If there was interaction between the two proteins, the signals in the spectra would be expected to shift or disappear on addition of one to the other. If there is no interaction, then there is no change in the position or number of signals. Although this is not a quantitative indication of how much two proteins interact, it is indicative of an interaction. From these NMR spectra (figure 25), it was clear that no significant changes are seen upon the addition of MupA to mmpD-ACP-4, which indicates there is not an interaction between these two proteins and that the transformation was not taking place on mmpD-ACP-4.

As 6-hydroxylation was not taking place on mmpD-ACP-4, the timing of this transformation was reconsidered. Another potential location for the 6-hydroxylation was the first ACP of *mmpA* (mmpA-ACP-1) as shown in figure 26.

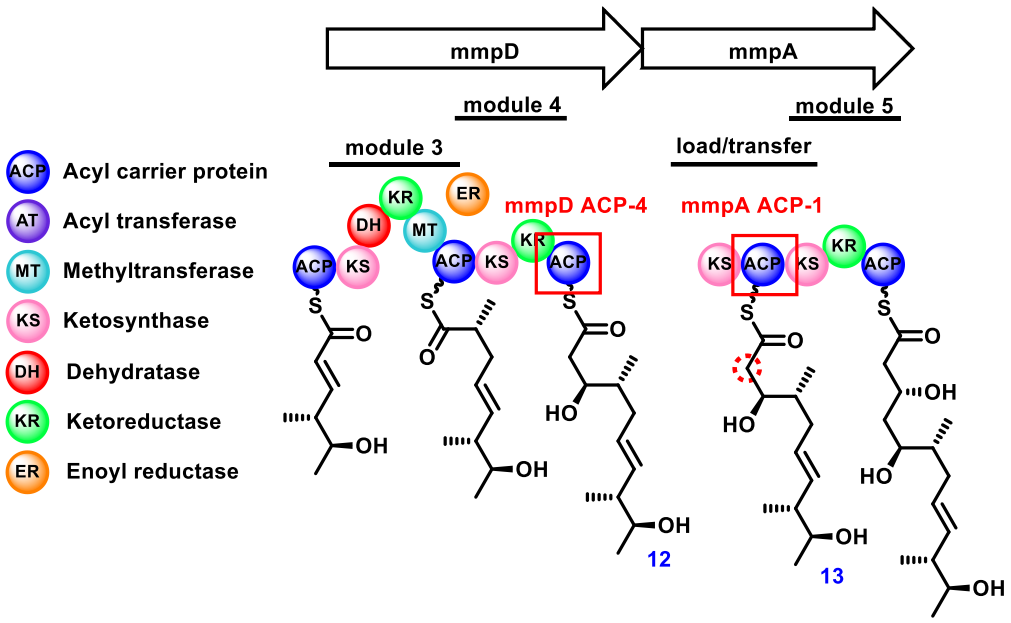


Figure 26. The final modules of *mmpD* and the first module of *mmpA*, with the two ACPs used in the experiments highlighted.

Before the upgrade of substrate **29** onto this ACP, an NMR assay of MupA with mmpA-ACP-1 was carried out by Dr Winter (figure 27). The spectrum in blue shows mmpA-ACP-1, while the spectrum in red was recorded after the addition of MupA. It can be seen that there are significant differences between the two spectra which is indicative of an interaction between mmpA-ACP-1 and MupA.

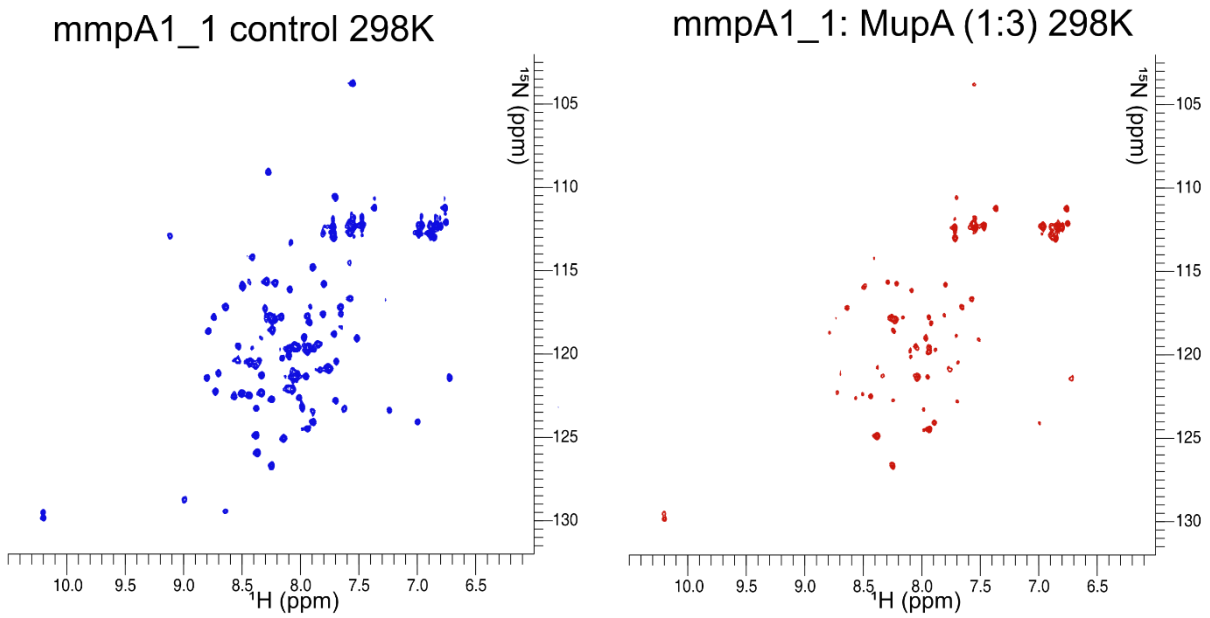
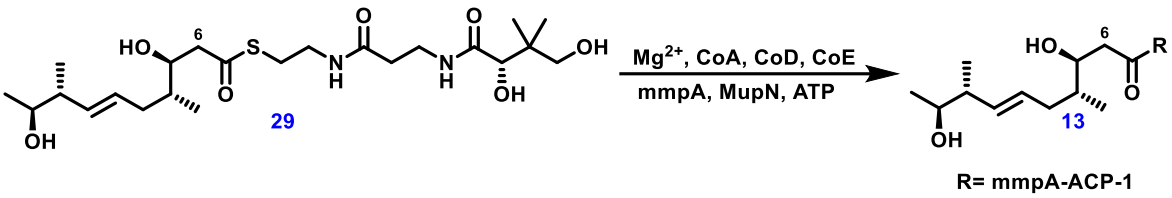


Figure 27. NMR assays of mmpA-ACP-1 (left) and after the addition of MupA (right) carried out by Dr Winter.

Encouraged by this positive interaction, substrate **29** was loaded onto this ACP by Dr Winter using the same conditions as discussed previously as shown in scheme 79.

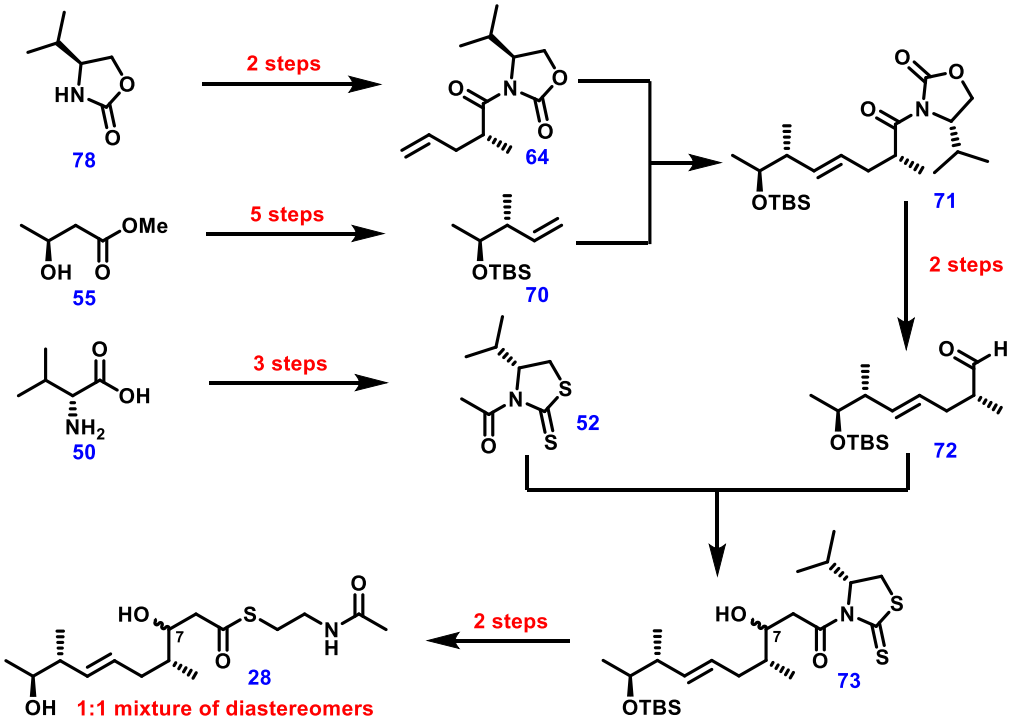


Scheme 79. The upgrade of substrate **29** to mmpA-ACP-1.

When bioassays were carried out by Dr Winter of substrate **13** with MupA, again no turnover by the enzyme was seen. Work is currently ongoing to determine exactly the conditions needed for this biotransformation.

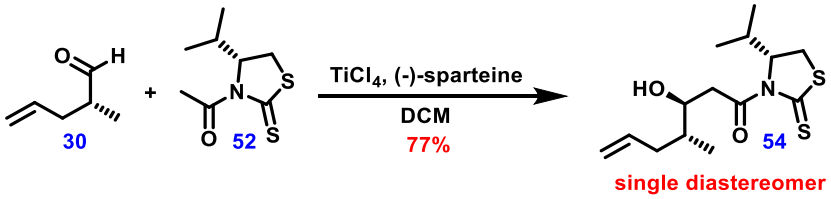
2.7 Conclusion

The total synthesis of thioester **28** was achieved in 11 longest linear steps and 4.9% overall yield albeit as a mixture of diastereomers epimeric at C-7. The key steps involved a cross metathesis of terminal olefins **70** and **64** to introduce the alkene bond with excellent control of the (*E*) double bond geometry. An aldol reaction installed the alcohol at C-7 and provided a handle for thioesterification.



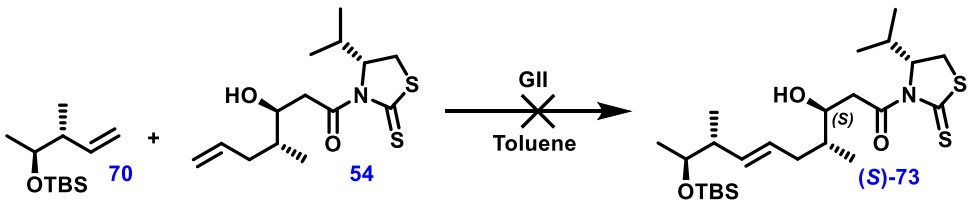
Scheme 80. The completed synthesis of **28**.

Model studies of the aldol reaction between aldehyde **30** and thiazolidithione auxiliary **52** were shown to proceed with complete stereocontrol (scheme 81), however when these conditions were applied to aldehyde **72**, a 1:1 mixture of diastereomers of unsaturated alcohol **73** was achieved. Although this was disappointing, the Felkin-Ahn transition state model, predicted selectivity for the opposite diastereomer to the one that was required and so perfect diastereoselectivity would be very difficult to achieve in this reaction.



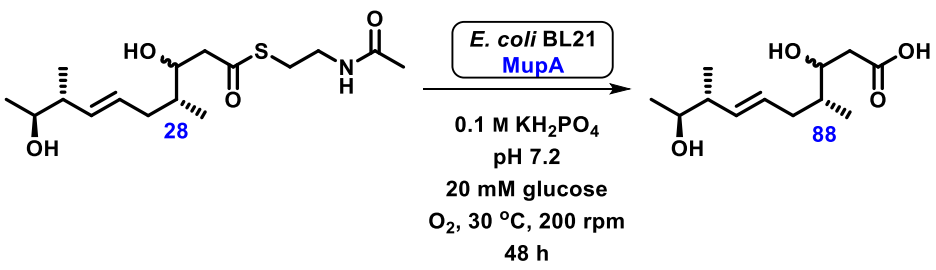
Scheme 81. Aldol reaction giving unsaturated alcohol **54** as a single diastereomer.

This synthetic route included a key cross metathesis step between terminal alkenes **70** and **64**, which was optimised and pleasingly gave the desired olefin in 58% yield. This cross metathesis did not take place in the presence of the thiazolidithione auxiliary, even under optimised conditions as shown in scheme 82.



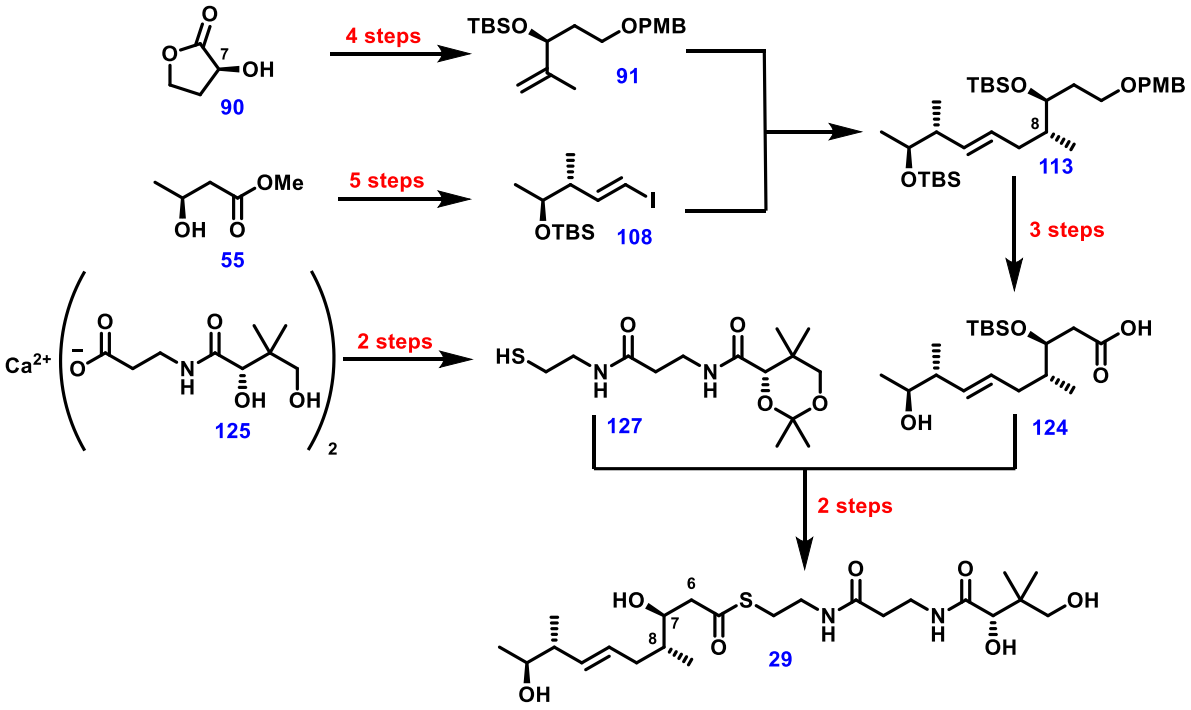
Scheme 82. The cross metathesis of **70** and **54** under optimised conditions.

Following the completion of the synthesis, bioassays of this substrate in *E. coli* expressing MupA were carried out, however the desired 6-hydroxylated material was not seen by HPLC and instead hydrolysis occurred to give acid **88**.



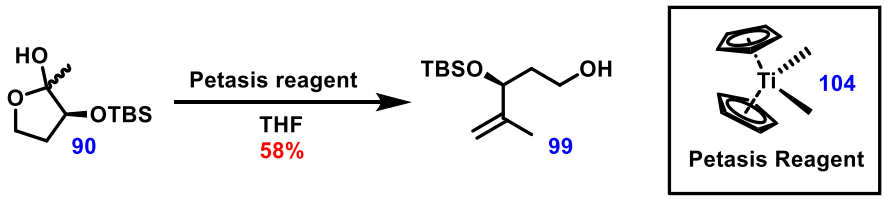
Scheme 83. Hydrolysis of thioester **28** to give carboxylic acid **88**.

The total synthesis of **29** was completed in 11 longest linear steps with 3.3% overall yield. The key disconnections included: a hydroboration which installed the stereocentre at C-8, a Suzuki cross coupling which installed the *E*-alkene, and a thioesterification which installed the pantetheine side-chain.



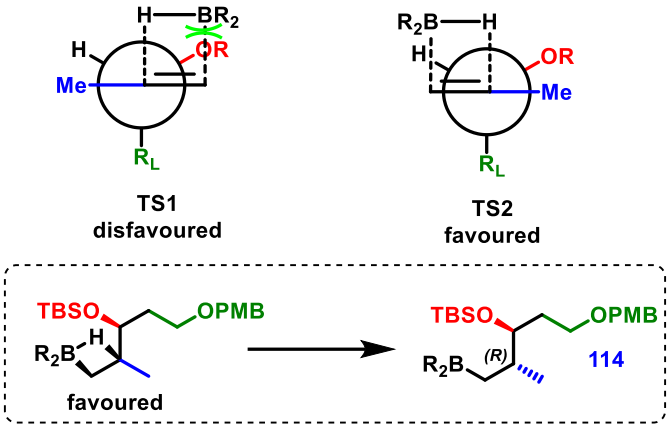
Scheme 84. Total synthesis of **29**.

After the initial difficulty in synthesising hydroxylactone **90**, it was purchased enabling the stereocentre at C-7 to be set from the starting material. The low yielding methylenation step was optimised by the use of Petasis reagent which gave alkene **99** in 58% yield as shown in scheme 85.



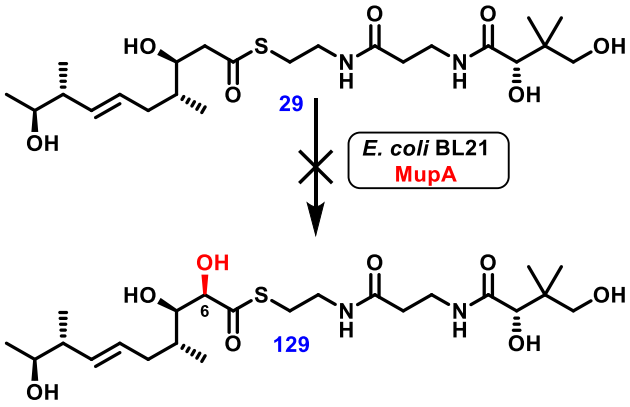
Scheme 85. The optimised methylenation to give **99**.

The hydroboration of unsaturated alcohol **91** gave a single diastereomer at the C-8 position, which was rationalised by applying Houk's modelling studies (page 57) to the two possible transition states as shown in scheme 86.



Scheme 86. Transition state models for the stereoselective hydroboration.

Following the final steps in the synthesis, substrate **29** was used in enzyme assays in *E. coli* expressing MupA however none of the required 6-hydroxylated material **129** was isolated as shown in scheme 87.



Scheme 87. The expected product **129** from bioassays with MupA.

Again, thioester **29** was hydrolysed to give carboxylic acid **88**. Due to the substrate not being turned over, it was decided to upgrade the substrate onto mmpD-ACP-4 as shown in scheme 77. Biotransformation studies were carried out by Dr Winter, however none of the desired product was observed by MS. An NMR assay of mmpD-ACP-4 and MupA showed no interaction, which led to the re-evaluation of the timing of 6-hydroxylation in the biosynthetic pathway. A similar NMR assay of MupA and mmpA-ACP-1 showed there to be an interaction between these two proteins and so substrate **29** was upgraded onto this ACP to give **13**. Biotransformation studies of substrate **13** with MupA showed none of the desired 6-hydroxylated to be present.

It is known that luciferase type monooxygenases have a low affinity for FMN and instead interact with the reduced form $FMNH_2$, which is why the presence of this species is crucial for the desired transformation. In order to achieve this reduced state, it is believed a co-reductase is acting, however at present this has not been identified in the mupirocin gene cluster. The reductase used in the experiments described in this chapter has been isolated from *E. coli*, however there is concern that this reductase is not working on this specific system. In order to probe this reductase further, work is ongoing to determine its activity level on NADH with different levels of aeration.

Work is currently being undertaken to determine whether oxygen concentration is inhibiting the process. In the presence of atmospheric levels of O_2, it is thought that the reduced $FMNH_2$ is being oxidised to FMN which is known to not interact with MupA, however O_2 is needed in order to form the Criegee intermediate and so work is currently being undertaken to carry out these bioassays in a carefully controlled atmosphere.

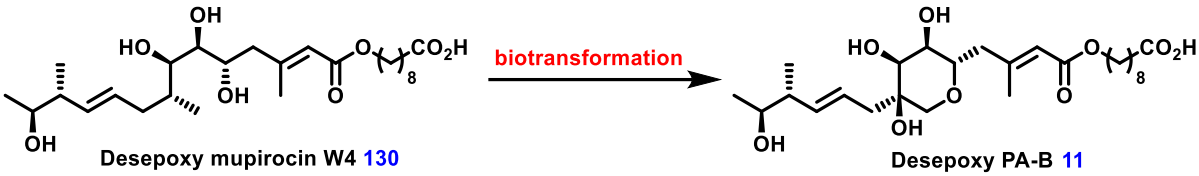
Another avenue of interest is the oligomeric state of MupA. Previous purification of MupA showed it to exist as a dimer, which is in agreement with other luciferase monooxygenases, however it has now been determined that this depends on concentration. At higher concentrations it has been noted that MupA exists as a tetramer, which is thought to inactivate MupA by making its active site inaccessible. Further work is needed to determine whether the concentration of MupA plays a role in the efficacy of the 6-hydroxylation. A crystal structure of MupA as a monomer or dimer would be highly valuable and to that end initial crystallography studies have commenced. By mapping the active site of MupA, more insight into the substrate that MupA acts upon could be provided.

CHAPTER 3: Investigating the specificities of MupW and MupZ

3. Chapter 3

3.1 Introduction

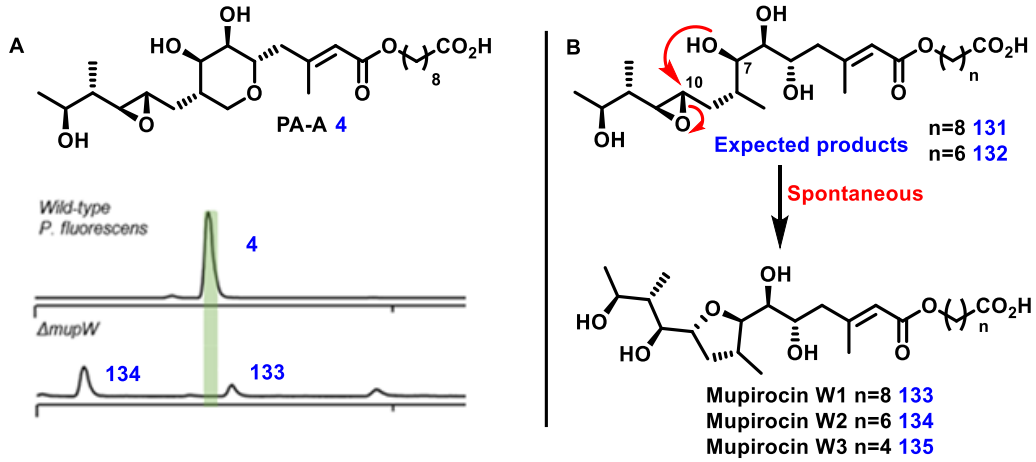
One of the most interesting transformations that takes place in the late stage biosynthesis of the pseudomonic acids is the formation of the tetrahydropyran (THP) ring from an unactivated methyl group, which would be very challenging to achieve synthetically. This transformation will be discussed in detail in this chapter.



Scheme 88. The proposed transformation of linear substrate **130** to desepoxy PA-B **11**.

3.1.1 Gene knockout experiments with the *ΔmupW* mutant

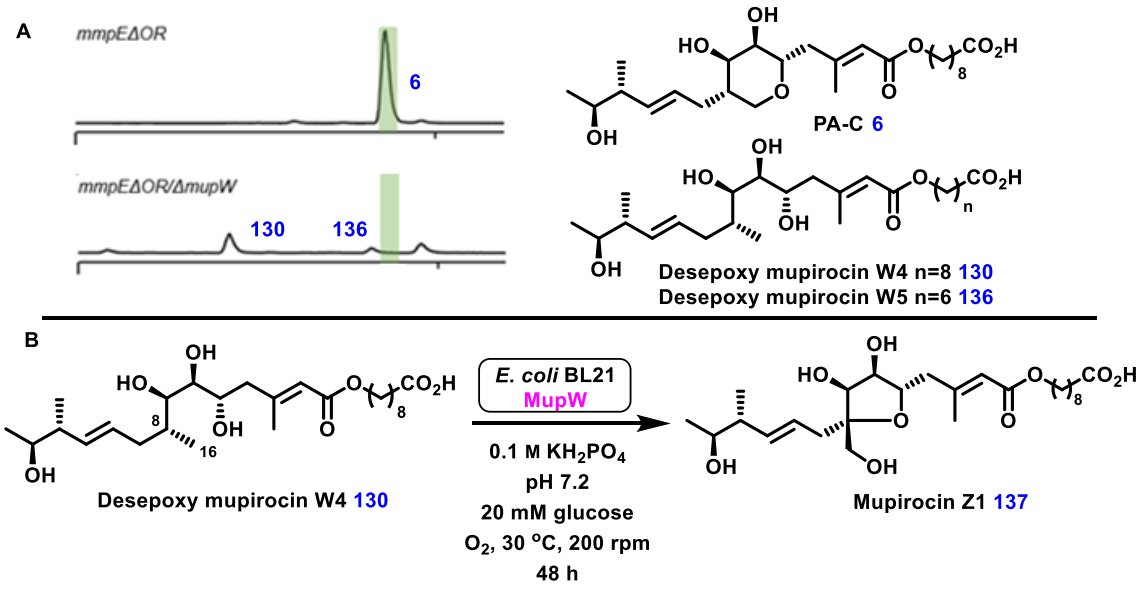
It was initially proposed that MupW, a Rieske non-haem oxygenase and its ferredoxin partner MupT, were responsible for the formation of the THP ring in the biosynthetic pathway.[36] *ΔmupW* gene knockout experiments in *P. fluorescens* gave mupirocins W1 **133** and W2 **134** as shown in scheme 89 B. It was hypothesised that these products arise from spontaneous intramolecular attack of the 7-hydroxyl group onto C-10, opening the epoxide and forming inactive bicyclic compounds as shown in scheme 89.[36]



Scheme 89. **A**: The structure of PA-A and HPLC trace of *ΔmupW* gene knockout experiments. **B**: Mupirocin W1 **133** and W2 **134** produced from the spontaneous cyclisation of expected products **131** and **132**.[36]

A fifth multifunctional modular protein *mmpEOR,* which acts to install the 10,11-epoxide, was subsequently identified.[19] It was hypothesised that gene knockout experiments with the *mmpEΔOR* mutant would produce metabolites lacking this epoxide and so would prevent the formation of the shunt products mupirocins W1 **133** and W2 **134** (scheme 89 B), while giving a more accurate insight into the function of MupW and MupT. Indeed, *mmpEΔOR* gene knockout experiments in *P. fluorescens* gave PA-C **6** rather than PA-A **4** as evident from the HPLC trace (scheme 90 A).[168]

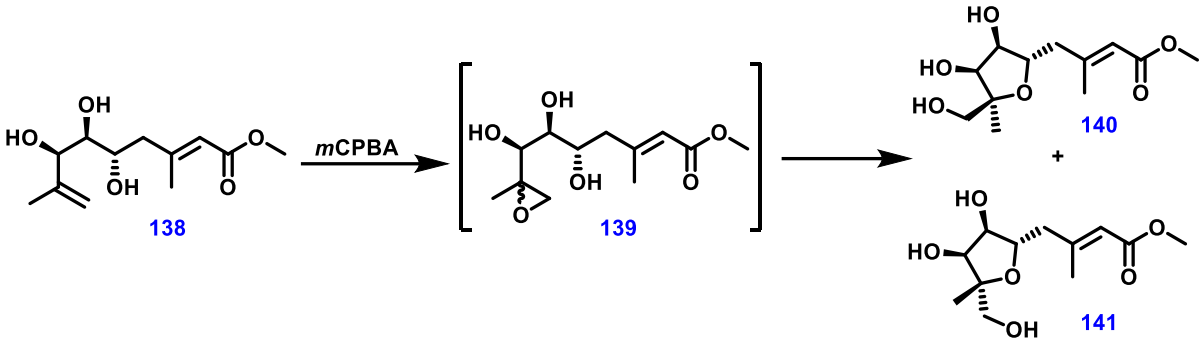
The double *mmpEΔOR/ΔmupW* mutant produced desepoxy mupirocin W4 **130** and W5 **136** in very low titres as shown in scheme 90 A, which supported the proposal that MupW was involved in the formation of the THP ring.[36] Desepoxy mupirocin W4 isolated from the *mmpEΔOR/ΔmupW* gene knockout experiment was incubated with *E. coli* expressing MupW, which led to the isolation of a product with the expected mass of the THP product desepoxy PA-B **11**, however structural elucidation by 1D and 2D NMR revealed that the product contained a five-membered ring, mupirocin Z1 **137** (scheme 90 B).[36]



Scheme 90. A: HPLC trace of *mmpEΔOR* gene knockout experiments producing PA-C **6** and *mmpEΔOR/ΔmupW* gene knockout experiments producing desepoxy mupirocin W4 **130** and W5 **136**. **B**: Isolation of THF mupirocin Z1 **137** from desepoxy mupirocin W4 **130**.

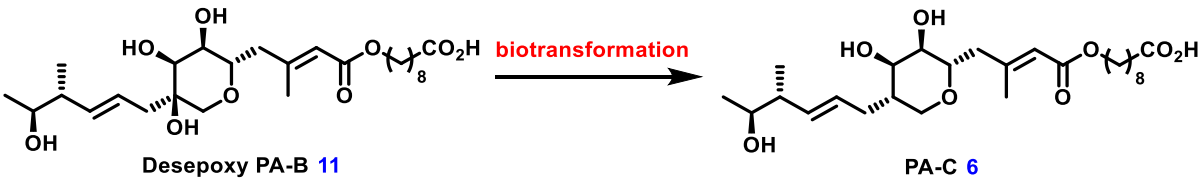
To gain further insight into the unexpected formation of this THF product **137**, Dr Bakar prepared alkene **138** for use in model studies (scheme 91).[168] When this alkene was reacted with *m*CPBA, no epoxide **139** was detected as it was rapidly transformed to the five

membered tetrahydrofurans **140** and **141** (scheme 91). Therefore, it was proposed that mupirocin Z1 **137** was being formed from spontaneous cyclisation of an 8,16-epoxide.[89, 168]



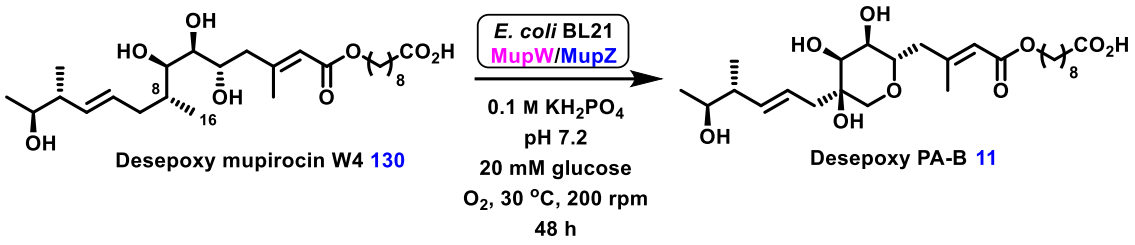
Scheme 91. Model studies gave two tetrahydrofurans **140** and **141**.[89, 168]

To investigate whether mupirocin Z1 **137** was an intermediate in the biosynthesis of the pseudomonic acids, it was fed to cultures of the *mmpEΔOR/ΔmupW* mutant of *P. fluorescens*, however only starting material was observed. In contrast, when desepoxy PA-B **11** was fed to cultures of this mutant it was transformed to PA-C **6** (scheme 92), which led to the conclusion that substrates bearing the THF ring are not intermediates in the pathway, but arise from spontaneous cyclisation of a proposed epoxide intermediate.[168]



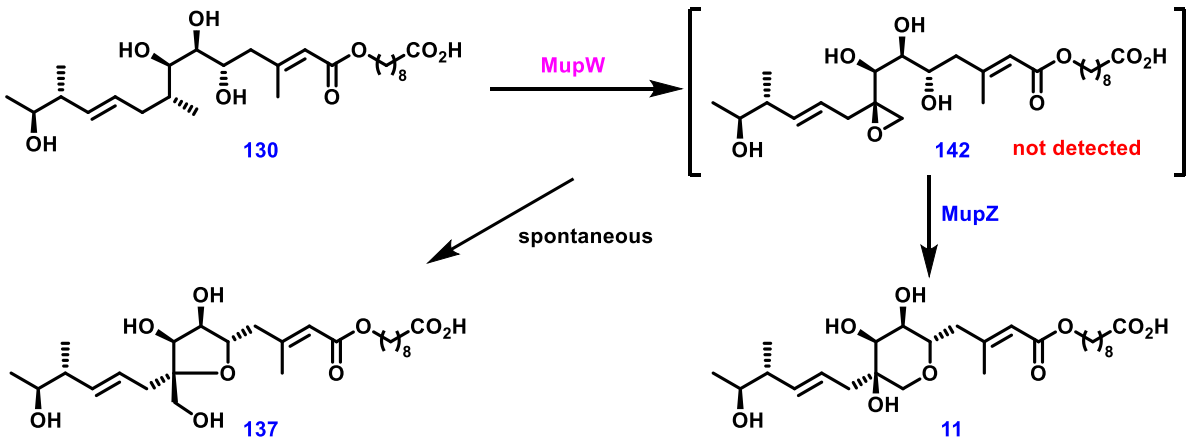
Scheme 92. Transformation of desepoxy PA-B **11** to PA-C **6** in cultures of *mmpEΔOR/ΔmupW* mutant of *P. fluorescens*.

It was therefore proposed that an epoxide is formed from the 8-methyl group of **130** catalysed by MupW, and that a second enzyme is required to effect 6-*endo* cyclisation to give THP **11**. It is important to note that there is much discussion over the use of '*endo*' in these types of cyclisation, as the bond breaking is outside of the newly formed ring. Another way of describing this is the use of 'fused' when describing the transition state, however both terms are used in the literature interchangeably.[169] From sequencing the mupirocin gene cluster, MupZ had not been assigned a function and so was proposed to have a role in this transformation as a possible epoxide hydrolase. Biotransformation studies were carried out with desepoxy mupirocin W4 **130** in *E. coli* expressing both MupW and MupZ, to determine whether MupZ was the missing epoxide hydrolase (scheme 93).



Scheme 93. Transformation of desepoxy mupirocin W4 **130** to desepoxy PA-B **11** catalysed by MupW and MupZ.[168]

Pleasingly this substrate **130** was turned over and desepoxy PA-B **11** was isolated by preparative HPLC and the structure confirmed by 1D and 2D NMR (scheme 93). These results demonstrated that MupW catalyses the oxidation of the unactivated 8-methyl group of **130**, while MupZ is an epoxide hydrolase which catalyses a 6-*endo*-tet cyclisation of a putative 8,16-epoxide **142** (scheme 94).[168]



Scheme 94. Proposed epoxide intermediate **142** undergoing spontaneous cyclisation to give THF **137** in the absence of MupZ, which catalyses the formation of the THP product **11**.

X-ray crystallographic studies were undertaken of MupZ, revealing that it is a symmetric homodimer (figure 28, A). The structure comprises five antiparallel β-pleated sheets augmented with three α-helices.[168]

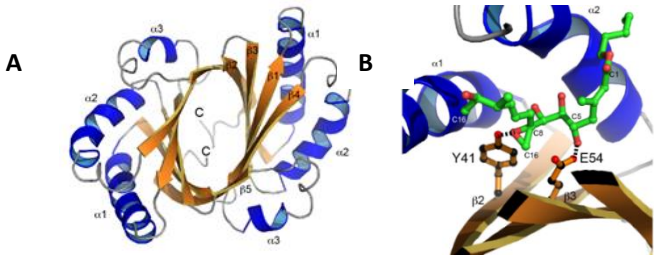
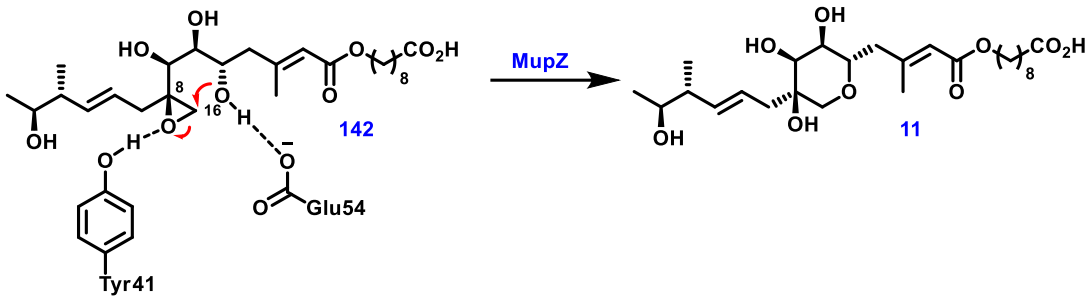


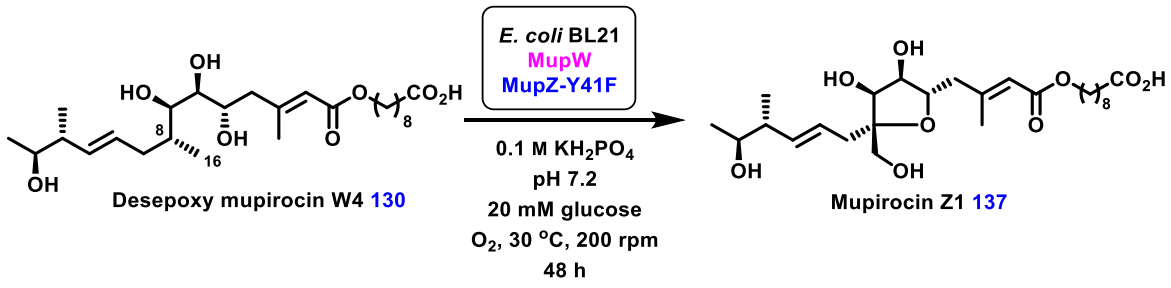
Figure 28. X-ray structure of MupZ (**A**) and a model of the active site of MupZ binding epoxide **142** (**B**).[168]

Molecular modelling studies were also undertaken on MupZ with docking of the proposed epoxide intermediate **142**. Two key amino acid residues in the active site, Tyr41 and Glu54 were identified. It was hypothesised that these are involved in an acid-base mechanism whereby Glu54 deprotonates the 5-OH, while Tyr41 protonates the epoxide oxygen, subsequently stabilising the developing transition state. In this way the desired THP ring product is produced as shown in scheme 11.[168] A model of this interaction is shown in figure 28 (B).



Scheme 95. The interaction of the acid base pair Tyr41 and Glu54 in the active site of MupZ.[168]

Mutagenesis experiments gave the point mutants MupZ-Y41F and Mupz-E54Q. Co-expression of MupW with either of the point mutants in *E. coli*, followed by incubation with desepoxy mupirocin W4 **130** led to the formation of the five-membered ring product **137** (scheme 96), in contrast to the THP product produced by WT-MupW/Z.[168] These results are in accordance with the proposed catalytic dyad involved in a 6-*endo* cyclisation of epoxide **142**.

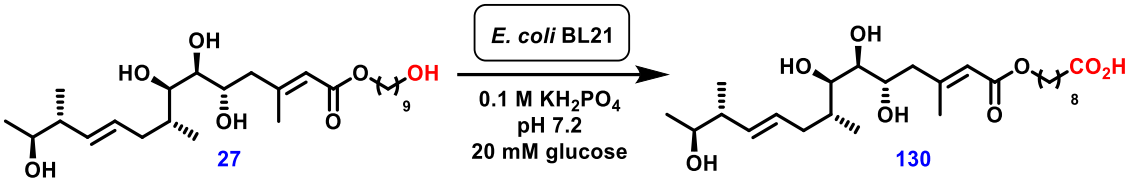


Scheme 96. Incubation of mupirocin W4 **130** with MupW and the point mutant MupZ-Y41F.[168]

Further studies are currently being undertaken to prove the existence of an epoxide intermediate and its mechanism of formation catalysed by MupW.

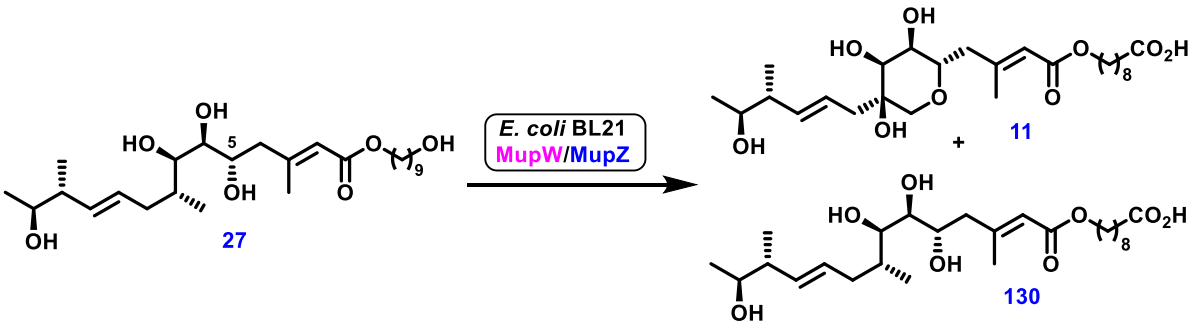
3.1.2 Previous substrate specificity studies on MupW and MupZ

To probe the substrate specificity of both MupW and MupZ, a number of substrates were used in whole cell bioassays with *E. coli* expressing MupW and MupZ, the products of which were analysed by LCMS and their structures elucidated by NMR.[89] It has been shown that unknown enzymes contained within the blank *E. coli* cells are responsible for the selective oxidation of the primary alcohol to the corresponding acid (scheme 97); this was fortuitous as it obviated the need to carry out this selective transformation chemically.



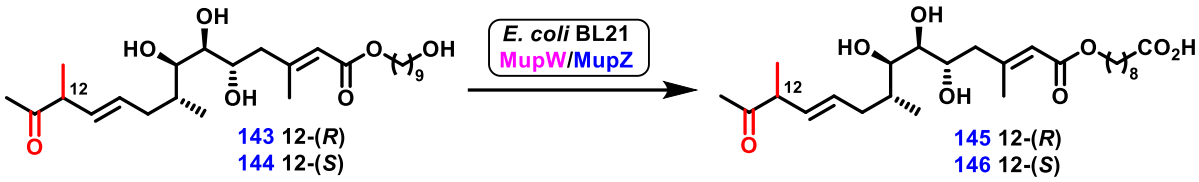
Scheme 97. Oxidation of desepoxy mupirocin W4-OH **27** by blank *E. coli* cells.[89]

Desepoxy mupirocin W4-OH **27**, synthesised by Dr Bakar**,** was incubated with *E. coli* co-expressing MupW and MupZ and was shown to be fully converted to desepoxy PA-B **11** and desepoxy mupirocin W4 **130** in a 3:1 ratio after 16 hours, which strengthened the working hypothesis that **130** is the precursor to desepoxy PA-B **11** (scheme 98).



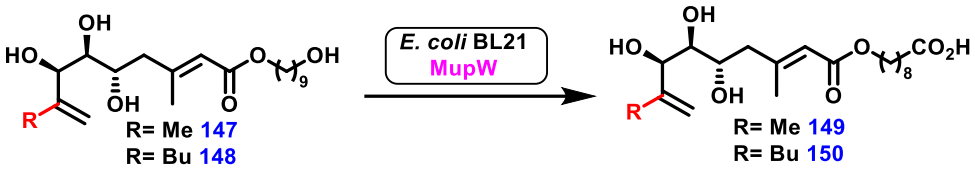
Scheme 98. Formation of THP **11** and acid **130** from incubation of desepoxy mupirocin W4-OH **27** with *E.coli* co-expressing MupW and MupZ.

Further studies have been aimed at investigating the substrate specificities of enzymes involved in this cascade process. Substrates **143** and **144**, synthesised by Dr Bakar, both bear a ketone at C-13 and **144** also has the unnatural (*S*) stereochemistry at C-12 (scheme 99). When these substrates were incubated in *E. coli* expressing MupW/Z, THP products were not observed by MS, although both substrates were oxidised to the corresponding acids **145** and **146** by *E. coli*.



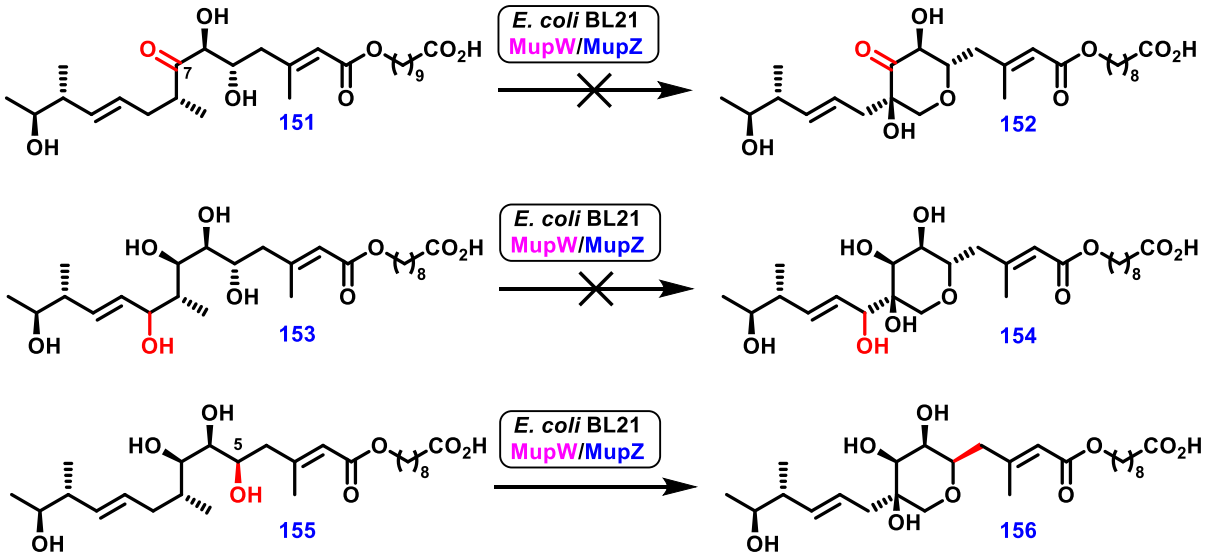
Scheme 99. Incubation of ketones **143** and **144** with MupW/Z in *E. coli* carried out by Dr L Wang.

When alkenes **147** and **148** with truncated side-chains, synthesised by Dr Bakar, were incubated with *E. coli* expressing MupW, both were oxidised to the corresponding carboxylic acids **149** and **150**, but no ring formation was apparent (scheme 100).[89]



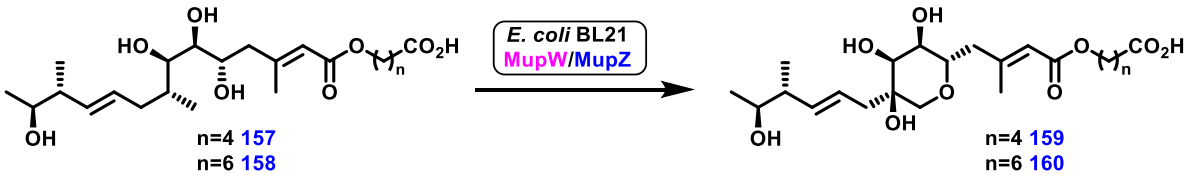
Scheme 100. Incubation of alkenes **147** and **148** *in E. coli* expressing MupW/Z carried out by Dr L Wang.[89]

The effect of changes to the structure of the core (C5-C9) of desepoxy mupirocin W4 **130** were also investigated. Ketone **151**, isolated from the ΔKR6 mutant,[54] alcohol **153**, isolated from the ΔDH4 mutant,[54,89] and alcohol **155** with unnatural stereochemistry at C-5, synthesised by Joe Barker, were incubated with *E. coli* expressing both MupW and MupZ, however only alcohol **155** was turned over to give the ring closed product **156**.



Scheme 101. Bioassays of ketone **151** and alcohols **153** and **155** with MupW/Z in *E. coli* carried out by Dr L Wang.

In addition, analogues of desepoxy mupirocin W4 with varying chain lengths (n=4,6), isolated from gene knockout experiments with the *ΔmupW* mutants, were also used in the biotransformations and pleasingly were all accepted and turned over by MupW and MupZ (scheme 102).



Scheme 102. Formation of THP **159** and **160** from incubation of analogues of desepoxy mupirocin W4 **157** and **158** with MupW and MupZ.

From these experiments it is clear that the biocatalytic system is very particular as to what structural modifications will be tolerated. Substrates modified in the C9-C14 portion were oxidised (primary alcohols to carboxylic acids) but not cyclised, whilst changes to the fatty acid chain length had no effect on the efficacy of MupW or MupZ. From experiments with the C-7 ketone **151** (scheme 101), it is hypothesised that a hydroxyl group is required at that position for formation of the THP ring to occur.

3.1.3 Project aim

The aim of this project was to prepare tetraol **161**, which retains all the structural features of desepoxy mupirocin W4-OH **27** except the 6-hydroxyl group. This was required to examine further the substrate specificities of MupW and MupZ as well as to give a new substrate with which to explore 6-hydroxylation.

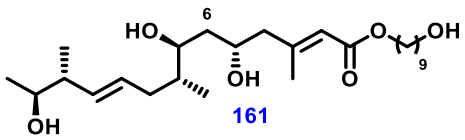
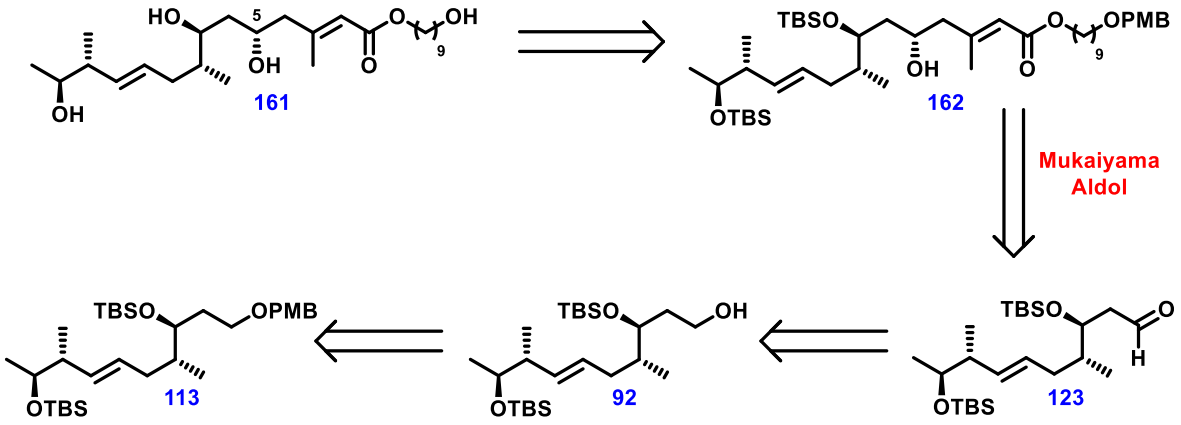


Figure 29. The structure of the target substrate **161**.

3.2 Retrosynthesis of tetraol 161 and vinylogous Mukaiyama aldol reactions

Having developed an efficient route to thioester **29** in the investigations involving MupA (chapter two), it was decided to apply elements of this route to the synthesis of substrate **161**. By utilising the same key Suzuki disconnection that was used previously in the synthesis of **29**, it was envisaged the route to substrate **161** could be modified to include a key

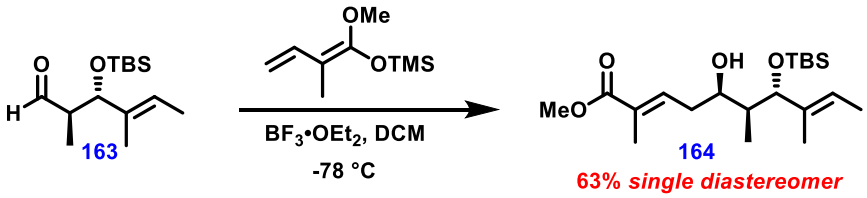
Mukaiyama aldol to install the fatty acid side chain, whilst also controlling the stereocentre at C-5.



Scheme 103. Proposed route for the synthesis of substrate **161** from protected triol **113**.

The synthesis of protected triol **113** will not be discussed in this chapter due to the detailed discussion of the synthetic approach to this key intermediate in chapter two.

Vinylogous Mukaiyama aldol reactions (VMARs) are valuable transformations in the synthesis of polyketides and allow large portions of polyketide backbone to be added to substrates, whilst also introducing a new stereogenic centre.[170-173] By reacting an aldehyde with an *O*-silylated ketene acetal in the presence of either a Lewis acid or a chiral ligand excellent stereoselectivity can be achieved. Scheme 104 shows an example of this carried out by Kalesse *et al*., utilising a boronate Lewis acid with excellent stereocontrol.[170]



Scheme 104. A vinylogous Mukaiyama aldol reaction carried out by Kalesse *et al*.[170]

The Lewis acid coordinates to the oxygen atom of the aldehyde making the carbon centre more susceptible to nucleophilic attack by the *O*-silylated ketene acetal. These species have been shown to be much more nucleophilic at the γ-position than the α-position, as opposed to metal dienolates, which are nucleophilic at the α-position (figure 30).[170]

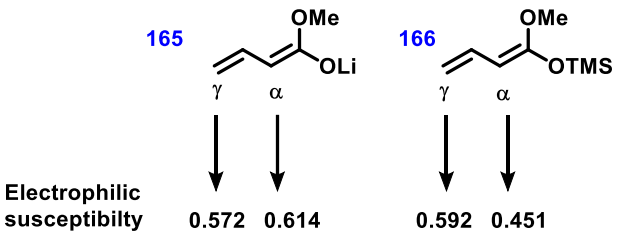
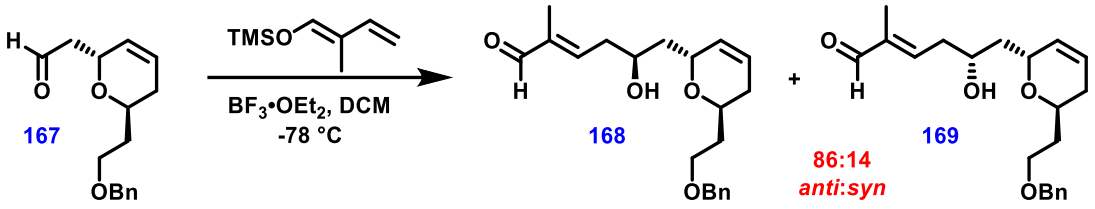


Figure 30. The electrophilic susceptibility of different positions in metal dienolates compared with *O*-silylated ketene acetals.[170]

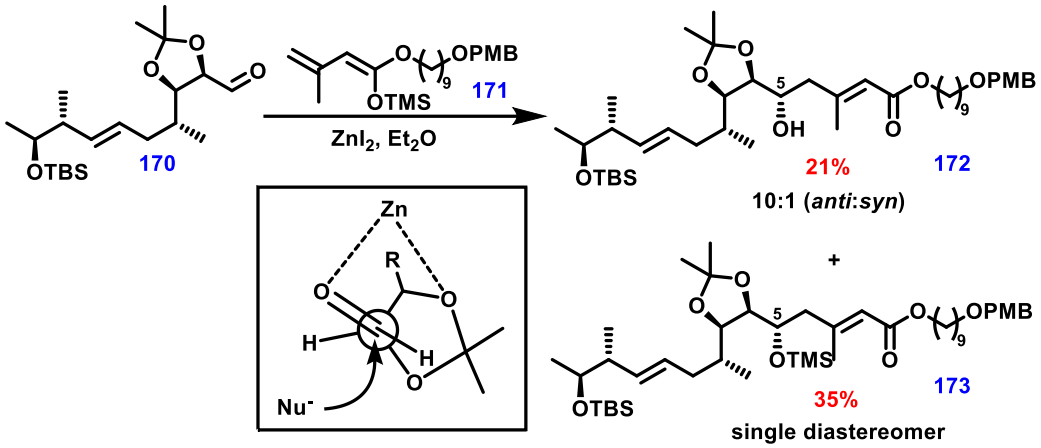
This was confirmed by using molecular orbital electron density calculations.[174] The HOMO coefficient and the electrophilic susceptibility value at the γ-position in *O*-silylated ketene acetal **166** is higher than at the α-position; a kinetic preference for the γ-addition product is therefore predicted. On the other hand, metal dienolate **165** displays a larger HOMO coefficient and electrophilic susceptibility value at the α-position than at the γ-position, and so the product of α-addition is favoured.

There is extensive literature precedent for selective 1,2-*syn* and *anti* VMARs through Felkin controlled addition of silyl ketene acetals to chiral α-substituted aldehydes.[175-180] Aldehyde **123** lacks an α-stereocentre, and so controlling the stereo-outcome of the Mukaiyama aldol reaction in the synthesis of tetraol **161** could prove to be challenging. There is limited literature precedence for the formation 1,3-*anti* products, however in the total synthesis of swinholide A, a polyketide derived antifungal agent, selectivity for the 1,3-*anti* product aldehyde **168** was achieved using $BF_3.OEt_2$ as the Lewis acid, with 72% *de*.[181]



Scheme 105. The VMAR in the total synthesis of swinholide A.[181]

In the total synthesis of desepoxy mupirocin W4-OH **27**, Bakar reported that silyl dienol ether **171** was successfully coupled to aldehyde **170** giving products **172** and **173** with high *de* under the conditions shown in scheme 106.[89]



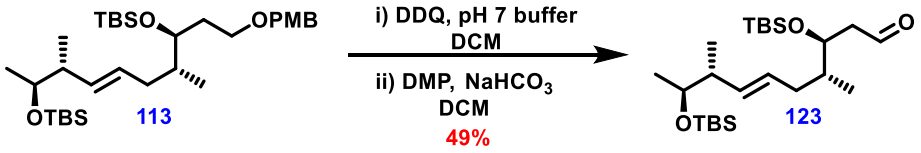
Scheme 106. The Mukaiyama aldol reaction carried out by Dr Bakar.[89]

The conditions for this reaction were first reported by Zhao *et al.* in the total synthesis of mupirocin H.[88] Zinc iodide is used as the Lewis acid as these conditions are mild enough to retain the acetonide protecting group and have been widely reported to give the desired stereochemical outcome in 1,2-directed vinylogous Mukaiyama aldol reactions.[88, 182] The stereochemistry of **172** and **173** can be rationalised by the transition state model shown in scheme 106. It is proposed that the zinc coordinates to the carbonyl oxygen and the C-7 oxygen in a 1,3- fashion. The Felkin-Ahn model shows that attack is then favoured at the *Si* face aldehyde **170**, generating the desired stereochemistry at C-5. These conditions could be applied to aldehyde **123** and silyl dienol ether **171** in the synthesis of substrate **161**.

3.3 Results and Discussion

3.3.1 Synthesis and coupling of aldehyde 123 and silyl dienol ether 171

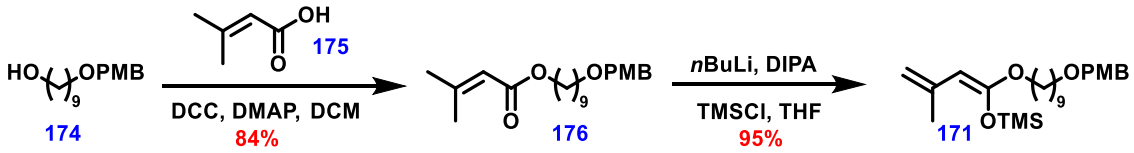
First, syntheses of the required coupling partners aldehyde **123** and silyl dienol ether **171** were carried out. Protected triol **113** was selectively deprotected using DDQ to give the primary alcohol, which was then oxidised with DMP to give aldehyde **123** (scheme 107).



Scheme 107. Synthesis of aldehyde **123**.

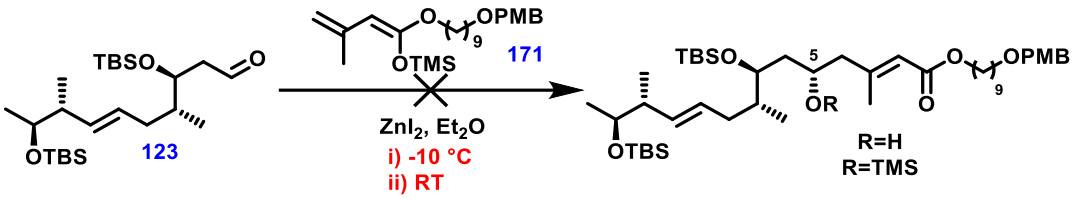
The synthesis of silyl dienol ether **171** began with the mono-protection of 1,9-nonanediol with PMBCl, NaH and TBAI in a DMSO/THF mixture to give alcohol **174**. This was coupled

with commercially available 3,3-dimethylacrylic acid **175** via a Steglich esterification[183] to give ester **176** in 84% yield (scheme 108). The synthesis of ester **176** was straightforward and high yielding, however formation of silyl dienol ether **171** proved challenging and irreproducible. Silyl dienol ether **171** was unstable on silica, and therefore with challenges associated with purification, it was vital to achieve full conversion of starting material **176** to product **171**. After optimisation, it was found that an excess of TMSCl and LDA was needed in order to give pure silyl dienol ether **171** in 95% yield.



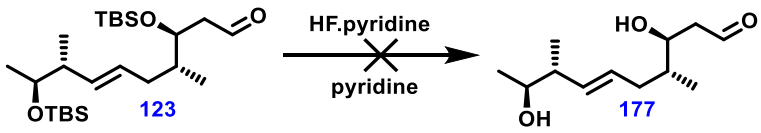
Scheme 108. Synthesis of silyl dienol ether **171**.

With both aldehyde **123** and silyl dienol ether **171** in hand, the VMAR between these coupling partners was investigated using zinc iodide at both -10 °C and RT (scheme 109), however, the reaction was unsuccessful and unreacted aldehyde **123** and ester **176** were isolated following column chromatography.



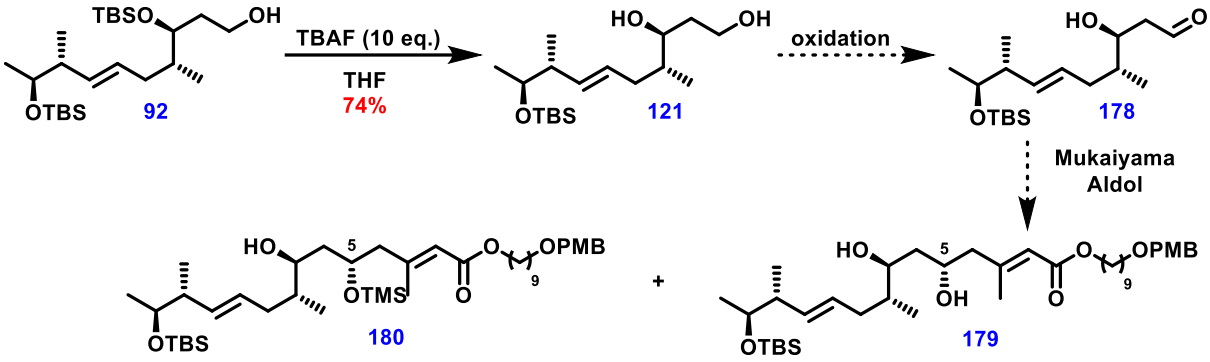
Scheme 109. Attempted Mukiayama aldol reaction of aldehyde **123** and silyl dienol ether **171**.

In the synthesis of desepoxy mupirocin W4-OH **27**, the 6,7-diol of aldehyde **170** had been acetonide protected (scheme 106), whereas in aldehyde **123** there was a TBS ether at C-7, hence it was hypothesised that the reaction was unsuccessful due to the bulk of this silyl protecting group. Thus it was deemed necessary to remove the silyl protecting groups of aldehyde **123**, however when this was attempted using HF.pyridine, degradation occurred (scheme 110).



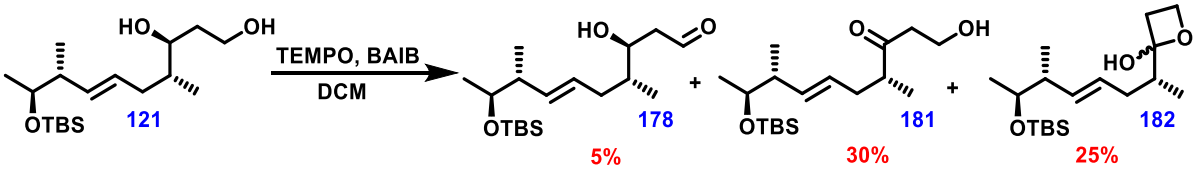
Scheme 110. The attempted deprotection of **123**.

A different deprotection strategy was investigated. Stirring alcohol **92** with 10 equivalents of TBAF for 24 h gave monoprotected triol **121**. It was proposed that the primary alcohol could be selectively oxidised to aldehyde **178**, which would then be used in a VMAR with silyl dienol ether **171** to give alcohol **179** and silyl ether **180** (scheme 111).



Scheme 111. Selective deprotection of silyl ether **92** gave alcohol **121**.

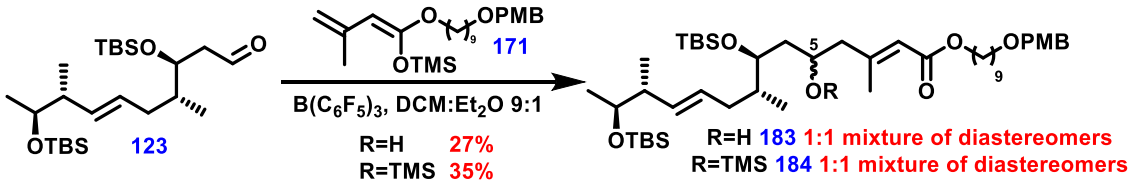
There is literature precedence for the selective oxidation of a primary alcohol in the presence of a secondary alcohol using TEMPO/BAIB as the oxidants.[184] When **121** was subjected to these conditions, a number of products were isolated as shown in scheme 112. The desired product aldehyde **178** was isolated in 5% yield, while the major product **181** was the result of oxidation of the secondary alcohol. In addition, an oxetane product **182** was also isolated, which is formed from attack of the primary alcohol into the ketone.



Scheme 112. Attempted selective oxidation of diol **121**.

Owing to the difficulties in synthesising aldehyde **178**, it was decided to return to the VMAR between aldehyde **123** and silyl dienol ether **171** using a different Lewis acid catalyst. Trispentafluorophenyl borane (TPPB) is a strong Lewis acid which has been shown to be compatible with silyl protecting groups, so this was used in the reaction as shown in scheme 113.[173] When aldehyde **123** and silyl dienol ether **171** were mixed with TPPB at -78 °C for 1 h, complete consumption of starting material was seen and a 1:1 mixture of C-5 epimers, **183** and **184** were isolated, which were inseparable by chiral, reverse and normal phase HPLC. The formation of the unnatural diastereomer was not surprising due to the difficulty

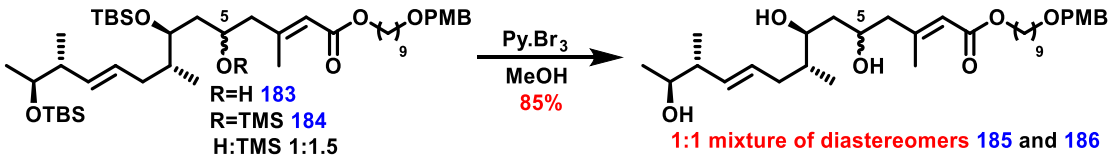
in controlling the stereochemistry as discussed in section 3.2 (page 83). However, its synthesis was fortuitous as feeding studies with the 'unnatural' epimer at C-5 had the potential to give greater insight into the specificities of MupW and MupZ, than feeding studies with tetraol **161** alone. To this end, the synthesis was continued with the hope that these diastereomers could be separated at a later stage.



Scheme 113. Mukaiyama aldol conditions to give alcohol **183** and silyl ether **184**, both as a 1:1 mixture of diastereomers.

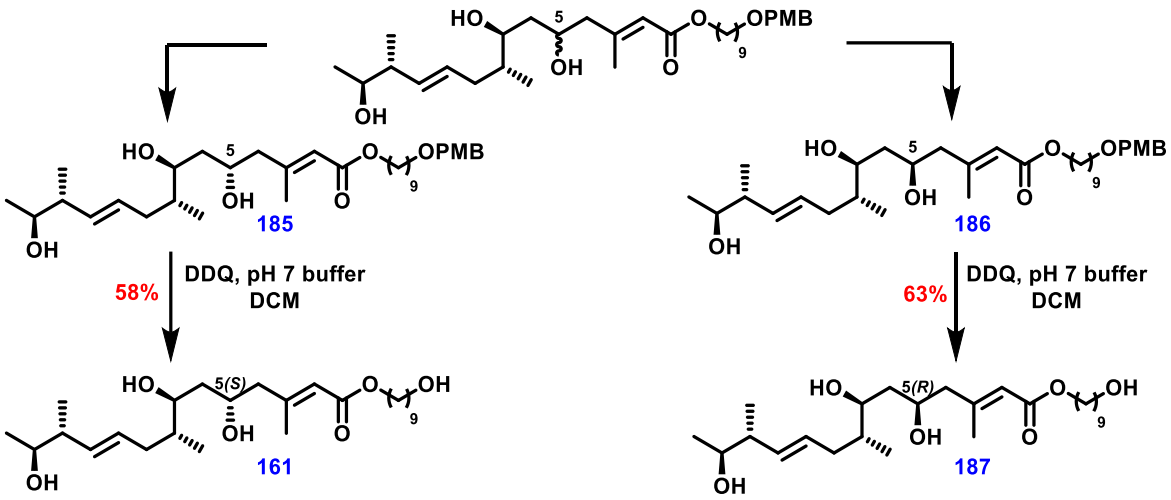
3.3.2 Completing the total synthesis of substrates 161 and 187

To complete the synthesis of the target tetraols **161** and **187**, it was necessary to remove the silyl protecting groups. The mixture of esters **183** and **184** were treated with 2 M HCl in THF, however this resulted in low yields and poor mass recovery of the required triols **185** and **186** as the expected mixture of diastereomers. Hence, **183** and **184** were treated with a catalytic amount of $Py.Br_3$ (0.1 equivalents) in MeOH which gave a 1:1 mixture of **185** and **186** in 85% yield as shown in scheme 114.



Scheme 114. Silyl deprotection of **183** and **184** to give a 1:1 mixture of diastereomers **185** and **186**.

The two diastereomers **185** and **186** were separated by column chromatography and deprotected with DDQ at pH 7 in DCM to give both tetraols with natural **161** and unnatural **187** stereochemistry at C-5 in 58% and 63% yield respectively (scheme 115).



Scheme 115. Separation of **186** and **187** followed by deprotection to give **161** and **187**.

At this stage it was not possible to conclusively assign the stereochemistry of each epimer, so it was decided to continue with the biotransformation studies with the hope that the stereochemistry could be assigned retrospectively by comparison of the products of these bioassays, to those previously isolated from *ΔmupA* knockout experiments (section 1.8.2). By comparing the NMR spectra (figure 31) of substrates **187** and **161**, the significant difference in shift of the 5-H and 7-H protons is evident.

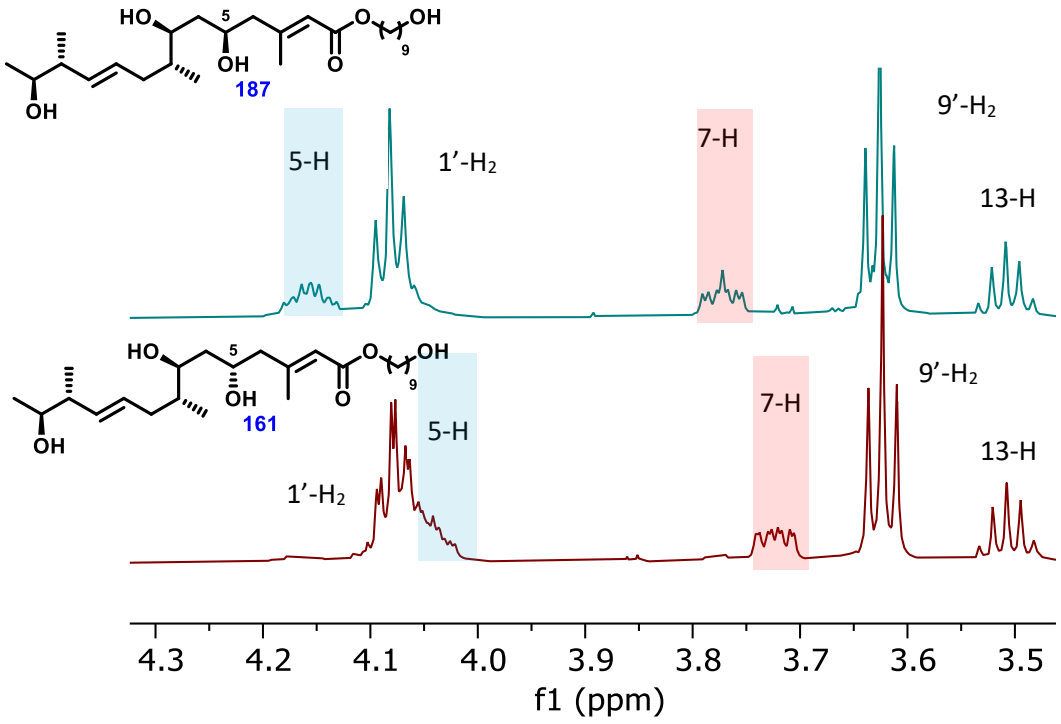
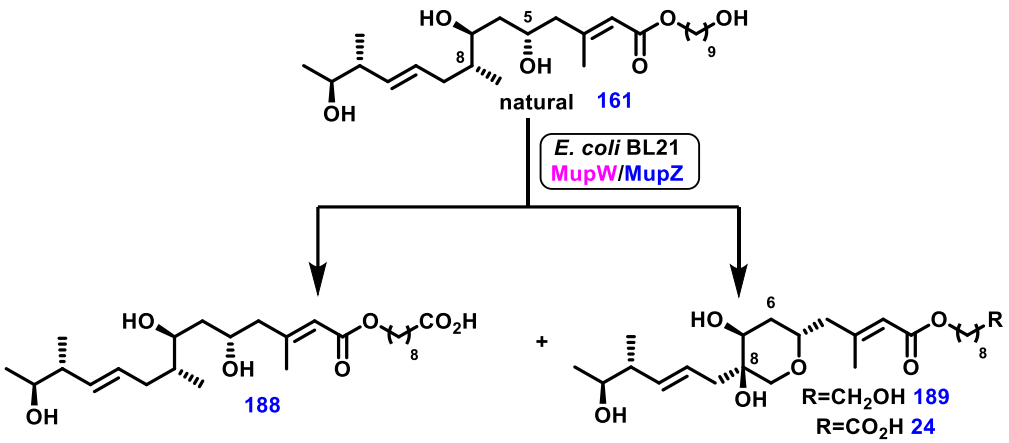


Figure 31. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of both diastereomers **187** and **161**.

3.4 Biotransformation studies of substrates 161 and 187 with MupW/Z in *E. coli*

The use of (un)natural in a scheme in this section denotes the (un)natural stereochemistry at C-5 compared to the natural product. With the two substrates in hand, biotransformation studies were carried out to determine the specificities of MupW and MupZ in *E. coli*. Pleasingly the primary alcohol of **161** was oxidised to acid **188** by *E. coli*, and the corresponding acid turned over after 24 h to give expected THP product **24** (scheme 116).



Scheme 116. Biotransformation of tetraol **161** to THPs **189** and **24** and acid **188**.

Interestingly, a small amount of THP **189** possessing the primary alcohol was isolated by preparative HPLC and the structure elucidated using 1D and 2D NMR. This result suggested that it is not necessarily a prerequisite for the primary alcohol to be oxidised before MupW and MupZ can act to form the THP ring. The expected THP **24** was also isolated by preparative HPLC and the structure elucidated by 1D and 2D NMR. Comparison of the ^{1}H NMR spectra of alcohol **161** with acid **188** (figure 32 A) showed there to be a disappearance of the protons α to the primary alcohol (1'-H_2) at δ 3.60 ppm and the appearance of a new triplet (2'-H_2) at δ 2.45 ppm consistent with an oxidation having occurred. To confirm the stereochemistry at C-5 and C-8 of substrate **161**, the ^{1}H and ^{13}C NMR data of THP **24** were compared with data from the same compound isolated from gene knockout experiments with the *ΔmupA* mutant as discussed in chapter one (page 25). Pleasingly, the data from both the ^{1}H (figure 32 A) and ^{13}C NMR (figure 32 B) for product **24** matched the spectra of the isolated metabolite, which validated our hypothesis that the hydroboration of alkene **91** (scheme 65, page 58) would give the (*S*)-stereochemistry at C-8 and that substrate **161** had the natural stereochemistry at C-5 (5*S*).

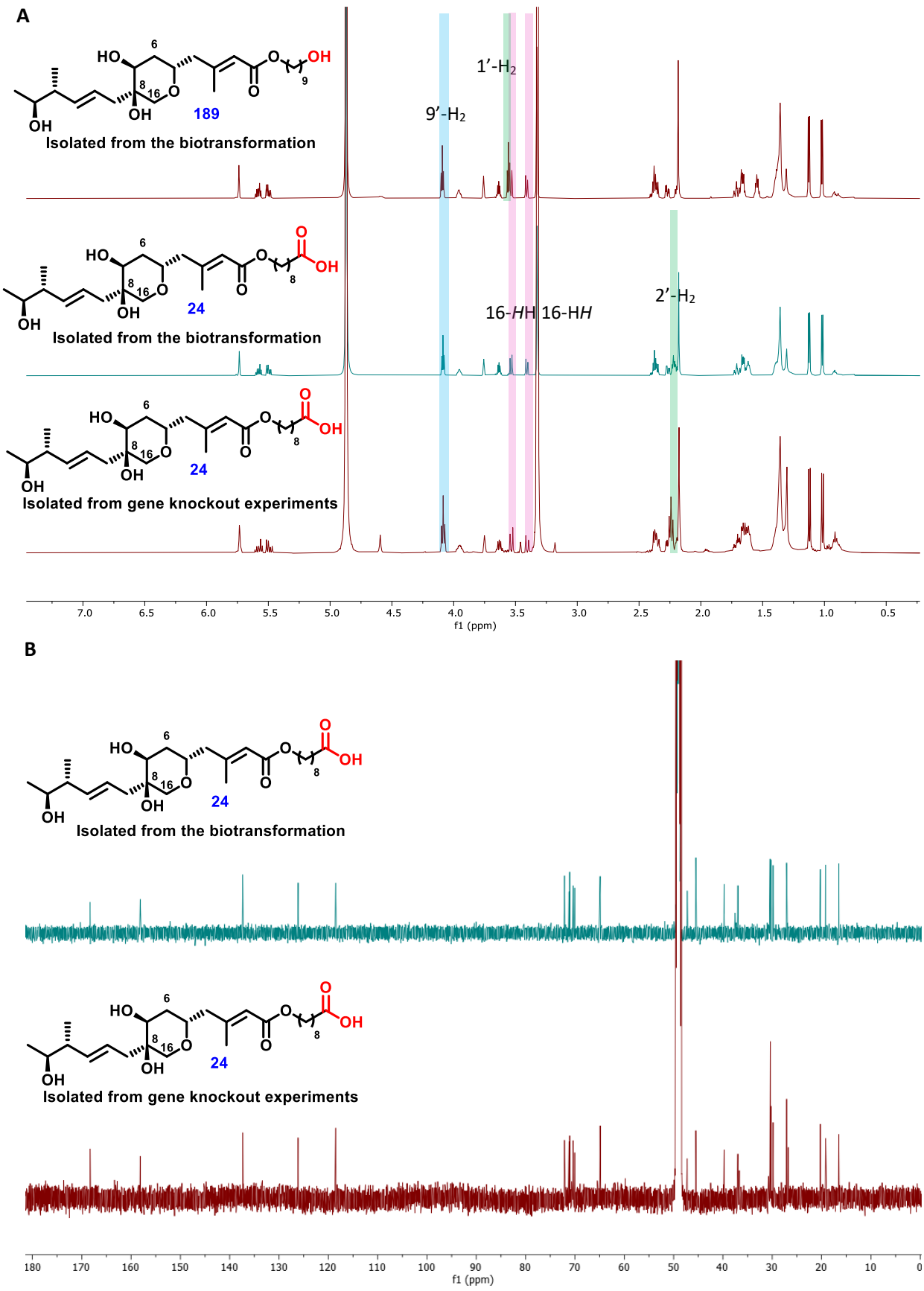
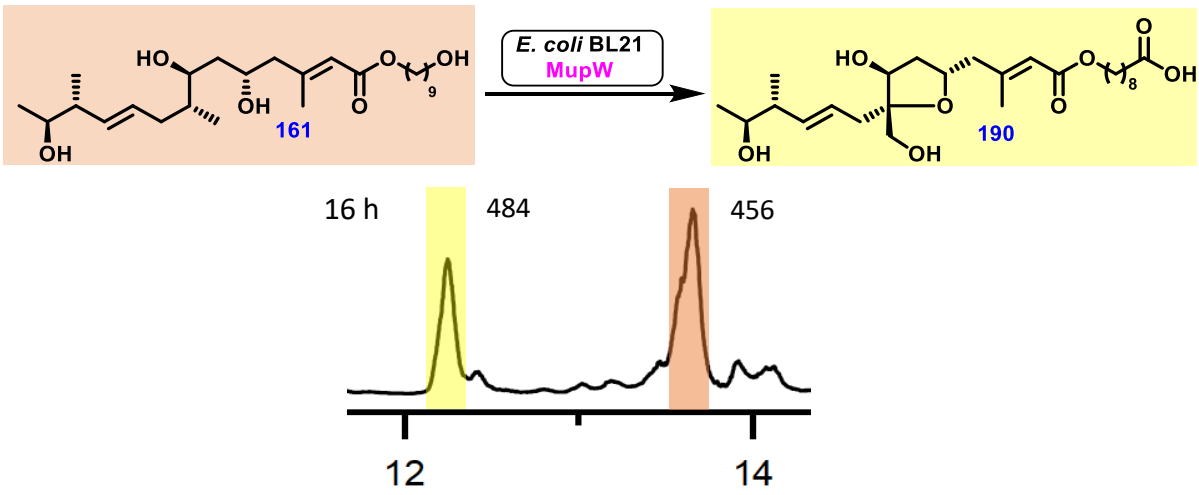


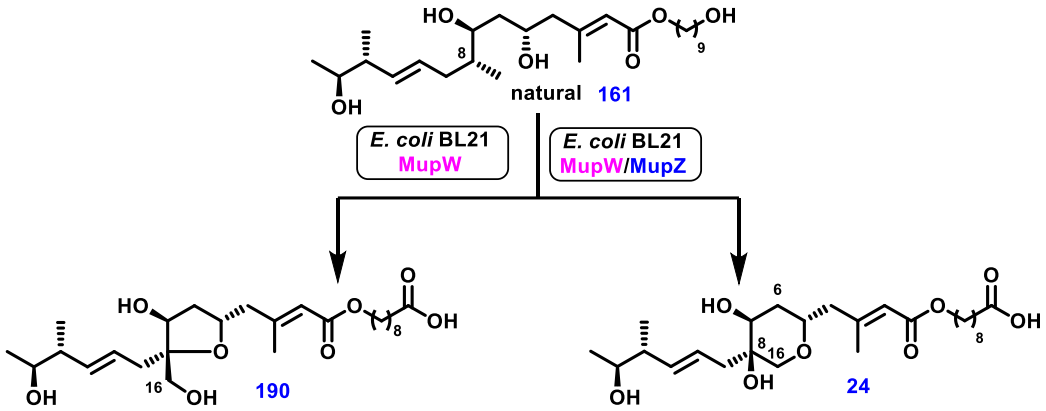
Figure 32. **A**: Comparison of isolated products **189** and **24** **B**: ^{13}C NMR of **24** compared with **24** isolated from gene knockout experiments with the *ΔmupA* mutant. (Both 700 MHz, CD_3OD).

The natural substrate **161** (*m/z* 456) was also incubated with *E. coli* expressing MupW, and pleasingly, the primary alcohol was oxidised, and ring closure occurred to give the expected THF product **190** with *m/z* 484 after 16 h (scheme 117). This product was isolated by preparative HPLC and the structure confirmed using 1D and 2D NMR.



Scheme 117. HPLC trace of the incubation of tetraol **161** in *E. coli* expressing MupW to give THF **190**.

Both the THF **190** and THP **24** products isolated from the biotransformations shown in scheme 118 have the same mass and so are difficult to differentiate between using mass spectrometry alone. In this way, NMR is the gold standard for identifying metabolites from biotransformations (figure 33).



Scheme 118. Biotransformation of **161** to THF **190** and THP **24**.

The disappearance of the methyl group at 0.90 ppm (8-CH_3) was the first indicator that a transformation had taken place (figure 33). Analysis of the HMBC and HSQC spectra of THP

24 showed there to be a correlation between 16-H_2 and 5-H, which gave evidence for the proposed structure being correct. No such correlation was seen in the 2D spectra of THF **190**.

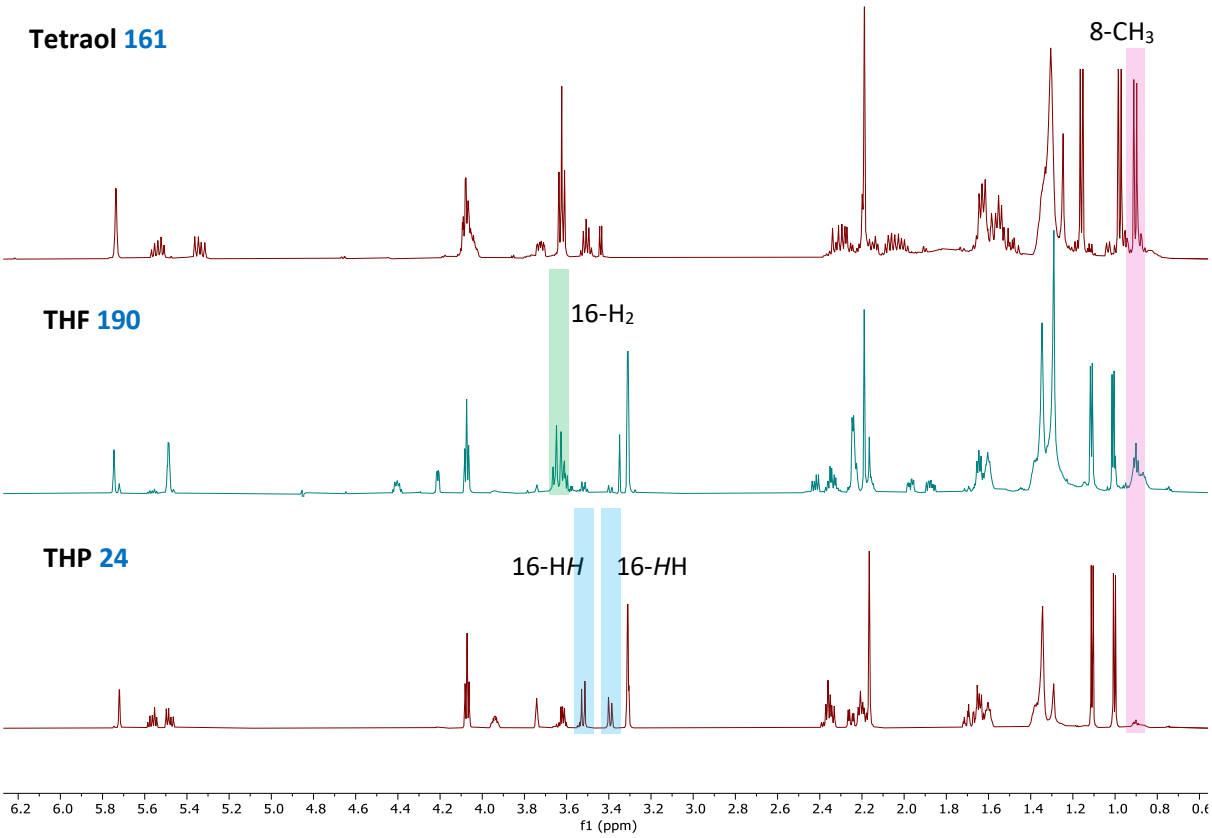
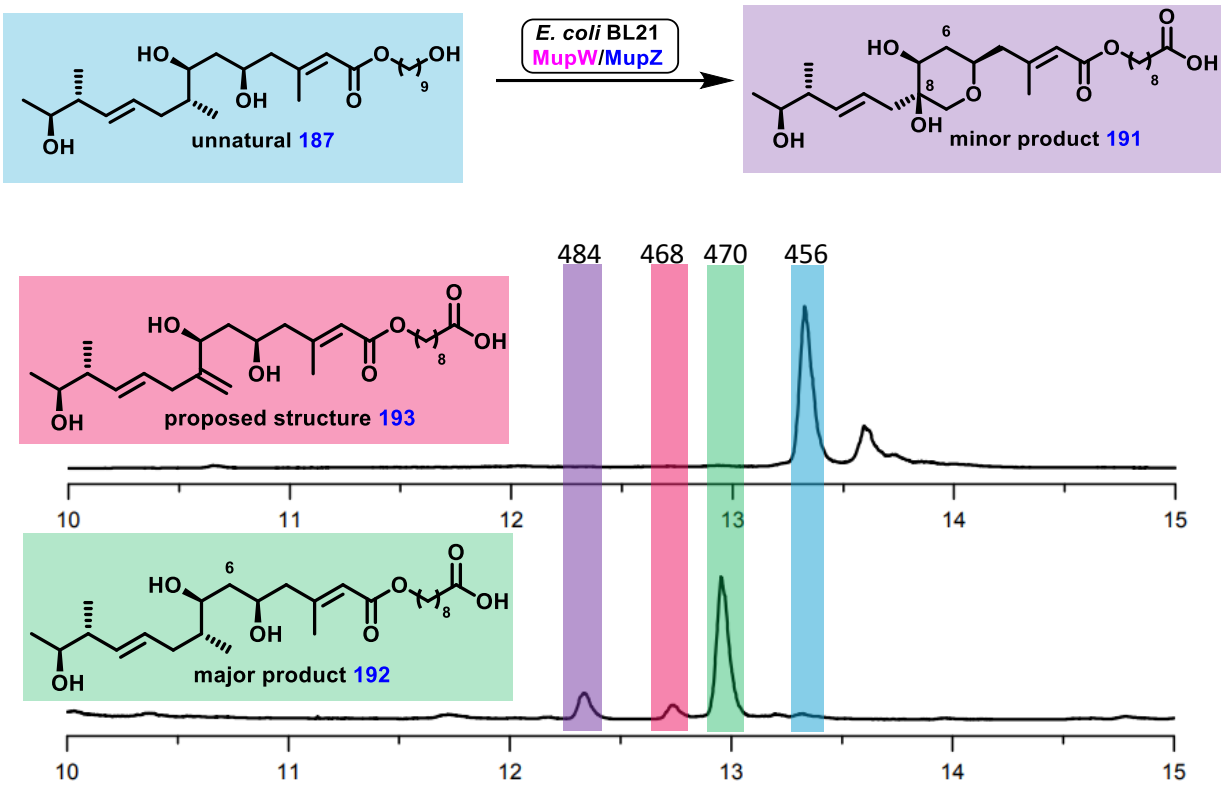


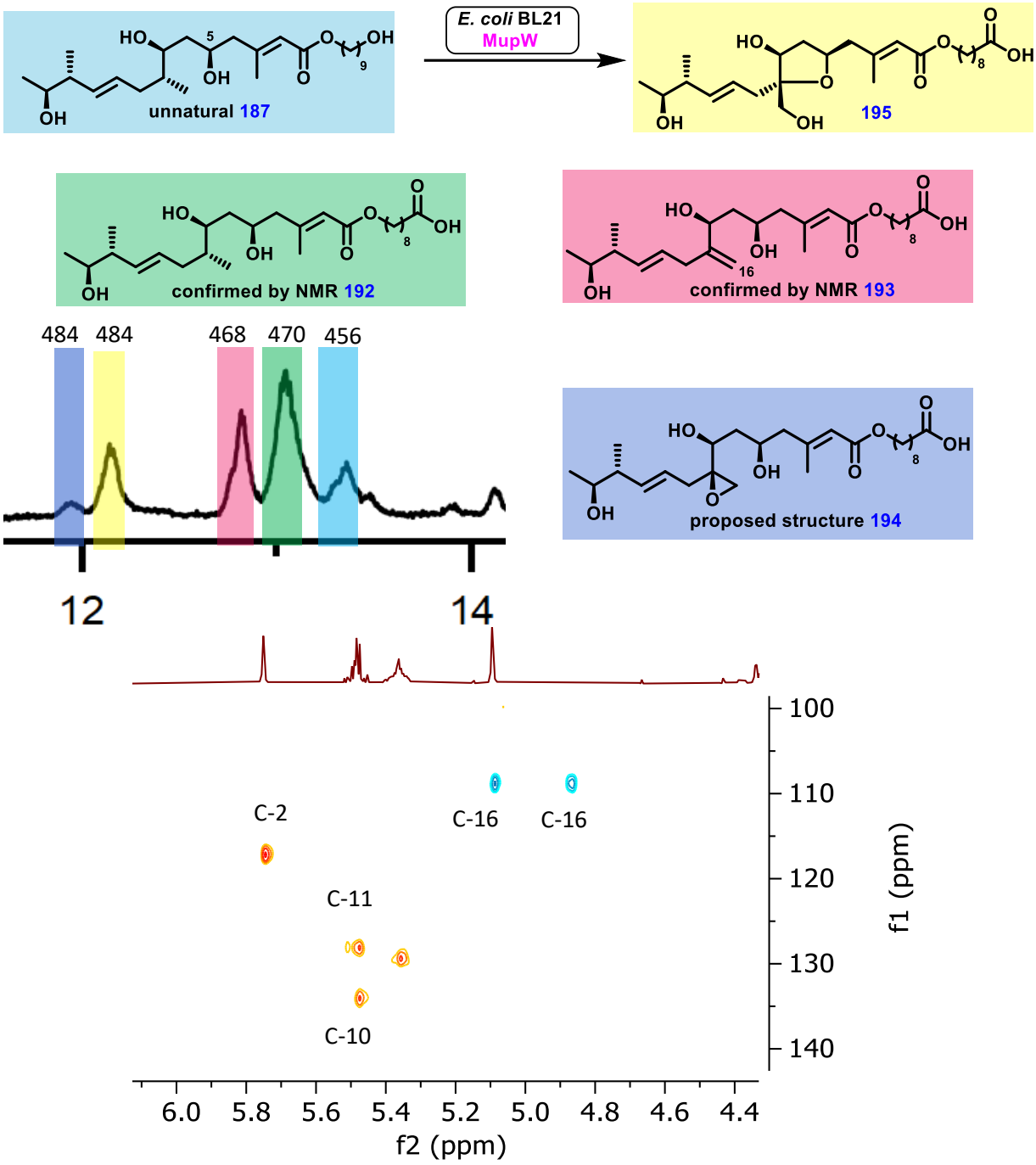
Figure 33. ^{1}H NMR spectra (700 MHz CD_3OD) of THF **190** and THP **24** isolated from the incubation of substrate **161** in *E. coli* expressing MupW or MupW/Z, compared with tetraol **161**.

Next the unnatural 5-epimer **187** was incubated with *E. coli* expressing MupW and MupZ (scheme 119). The major product after 24 hours was the product of oxidation, carboxylic acid **192**. Two further peaks were apparent by HPLC and each was isolated. The peak with a retention time of 12.4 mins had *m/z* 484 and was tentatively assigned as cyclic product **191**, while the peak with a retention time of 12.9 mins gave mass 468 and was tentatively assigned as alkene **193**, however there was insufficient material of both, for them to be isolated and characterised by NMR spectroscopy.



Scheme 119. Biotransformation of **187** to **191** and HPLC trace of this transformation.

It was proposed that alkene **193** may be an intermediate to epoxide **194** generated by MupW, whilst MupZ would catalyse the cyclisation of this intermediate to the THP. Hence a biotransformation was carried out in *E. coli* expressing MupW alone to support this proposal. Unnatural (5*R*) substrate **187** was incubated in *E. coli* expressing MupW and this biotransformation analysed by HPLC after 16 h (scheme 120). Interestingly, five compounds with different retention times were observed by HPLC, including the expected THF product **195** (scheme 120). Again, a peak with mass of 468 was seen, which pleasingly was isolated by preparative HPLC and confirmed to be alkene **193** by 1D and 2D NMR. Initially, it wasn't clear whether this isolated metabolite was the proposed 8,16-alkene due to the presence of only one new alkene proton in the ^{1}H NMR spectrum. However, analysis of the 2D NMR data (scheme 120) showed there to be two new methylene protons. The missing proton in the ^{1}H NMR spectrum was underneath the solvent peak at δ 4.85 ppm.

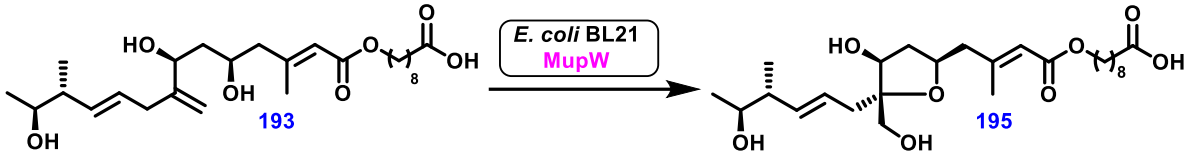


Scheme 120. HPLC trace of the biotransformation of **187** to **195**. HSQC (presat CD_3OD) showing the presence of two new methylene carbons.

Another interesting result from this biotransformation (scheme 120) was the appearance of a peak with a mass of 484 and a retention time of 11.9 mins, which could correspond to epoxide **194**, a putative intermediate in the formation of the THP ring. This epoxide is highly reactive and has never been observed or isolated by HPLC, and so confirming its existence by NMR would prove our proposed mechanism of THP formation to be correct. Work was

undertaken to isolate this metabolite by preparative HPLC, however the amount of material isolated proved too little to elucidate the structure by 1D and 2D NMR spectroscopy.

Alkene **193** isolated from the biotransformation shown in scheme 120 was re-fed to *E. coli* expressing MupW, with the expectation it would be transformed to THF **195**, and pleasingly this was observed by HPLC. This lends evidence to our hypothesis that in mupirocin biosynthesis, the 8-methyl group is converted to an alkene which is then epoxidised prior to cyclisation to form the THP product.

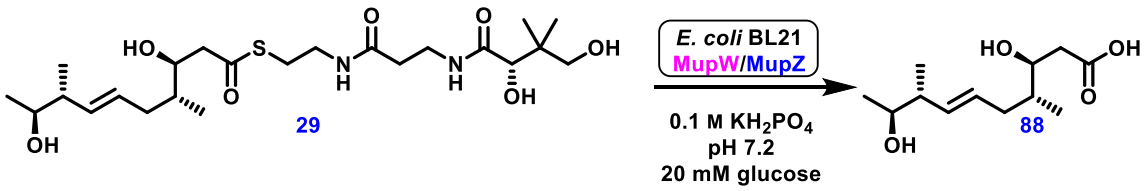


Scheme 121. Biotransformation of 8,16-alkene **193** to THF **195**.

The combination of these results proved that not only are substrates lacking the 6-hydroxyl group well tolerated by both MupW and MupZ, but also the unnatural stereochemistry at C-5 (**187**) is accepted. From feeding studies with unnatural (5*R*) substrate **187**, it was possible to isolate a range of different metabolites including alkene **193**, which we have proven is an intermediate in the formation of the THF ring **195**.

3.4.1 Biotransformation studies of thioester 29 with MupW and MupZ

Pantetheinic substrate **29**, synthesised in chapter two for the MupA project, was incubated in *E. coli* co-expressing MupW and MupZ, the purpose of which was to determine whether the MupW/Z system would recognise substrates with a different side chain from the fatty acids found in the pseudomonic acids.

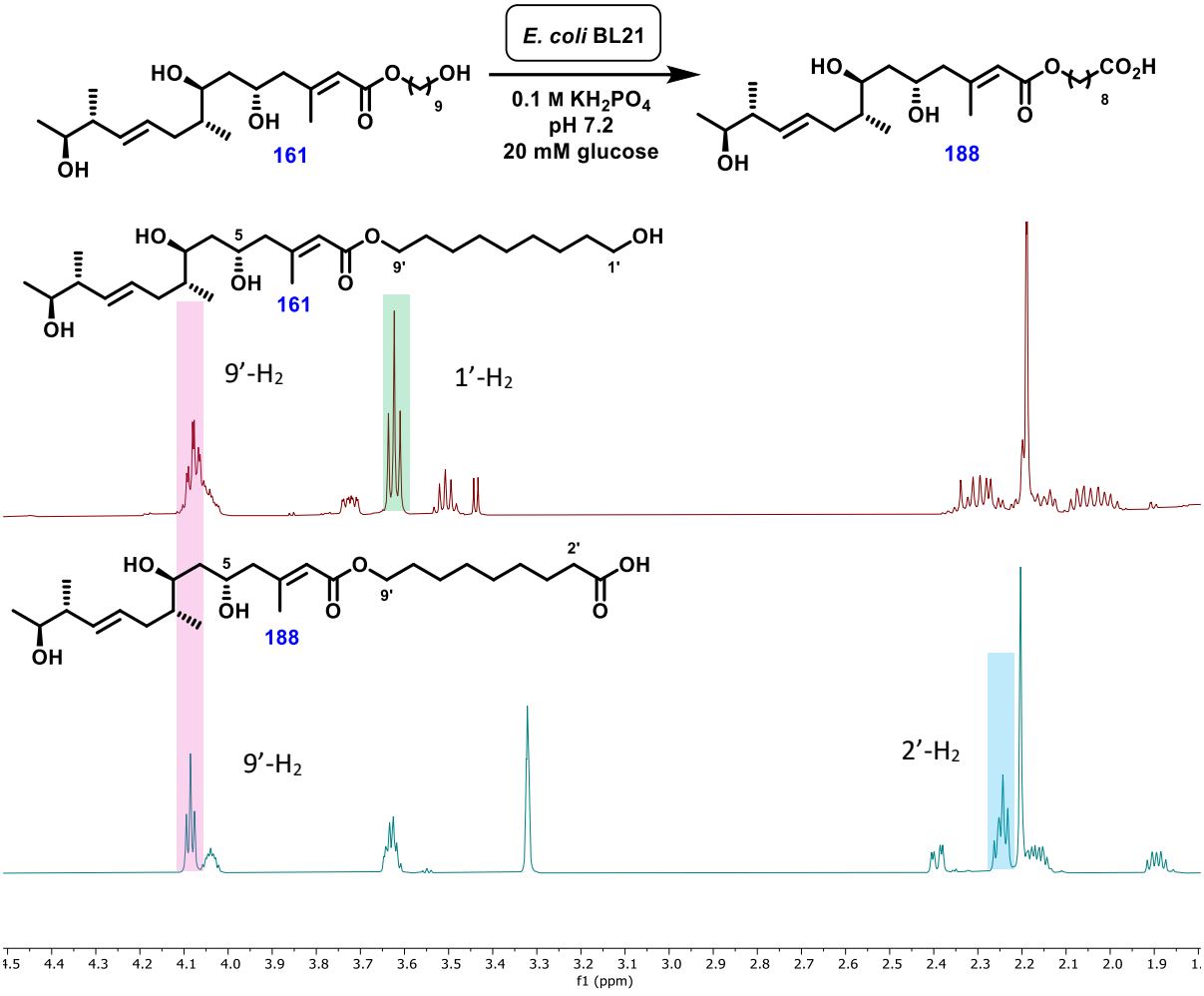


Scheme 122. Biotransformation of thioester **29** to acid **88**.

The only product observed arose from thioester hydrolysis giving the corresponding acid **88** (scheme 122). It is known that this acid **88** is not a substrate for MupW.

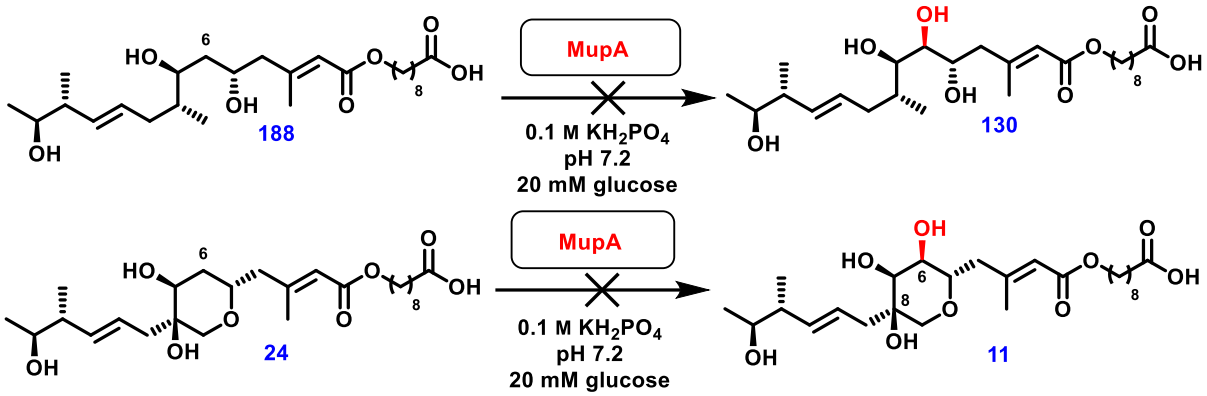
3.4.2 Biotransformation studies of substrates 188 and 24 with MupA

Next, studies were undertaken with natural substrate **161** to probe whether the proposed 6-hydroxylase, MupA (discussed in chapter two), would act on a substrate bearing the full fatty acid side chain. Tetraol **161** was incubated with blank *E. coli* cells in order to access the corresponding acid **188** for enzyme assays with MupA. This was necessary as these enzyme assays were not conducted in *E. coli*, and so substrate **161** would not otherwise have been oxidised. The ^{1}H NMR of **161** showed a triplet at δ 3.60 ppm assigned to 1'-H_2, whereas in acid **188**, this signal had disappeared and a new signal was apparent at δ 2.25 ppm assigned to 2'-H_2.



Scheme 123. Oxidation of alcohol **161** to acid **188** by *E. coli*.

Acid **188** was isolated by preparative HPLC and incubated with MupA to determine whether 6-hydroxylation would occur, however analysis by HPLC showed no new peaks and the starting material was returned unchanged.

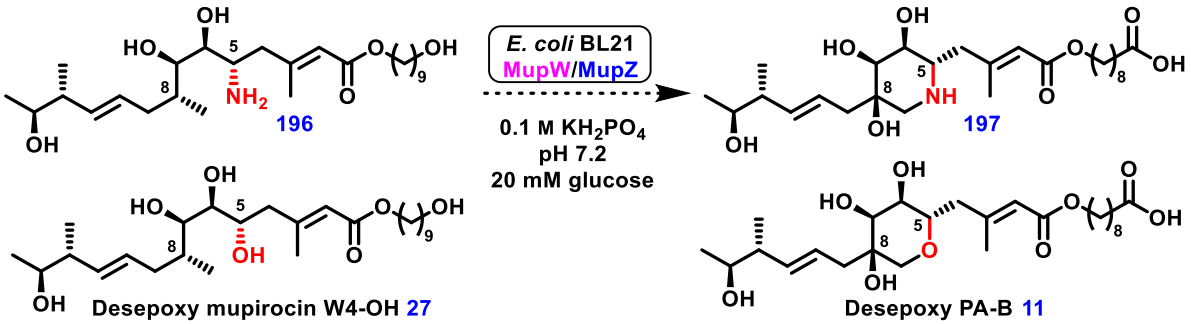


Scheme 124. Biotransformation studies carried out with substrates **188** and **24** with MupA.

In addition, THP **24**, isolated from the incubation of substrate **161** with *E. coli* co-expressing MupW and MupZ (scheme 124), was incubated with MupA. Analysis by HPLC showed no turnover had taken place, which lends further evidence to the hypothesis that 6-hydroxylation takes place prior to cyclisation and installation of the fatty acid side chain, as discussed in chapter two.

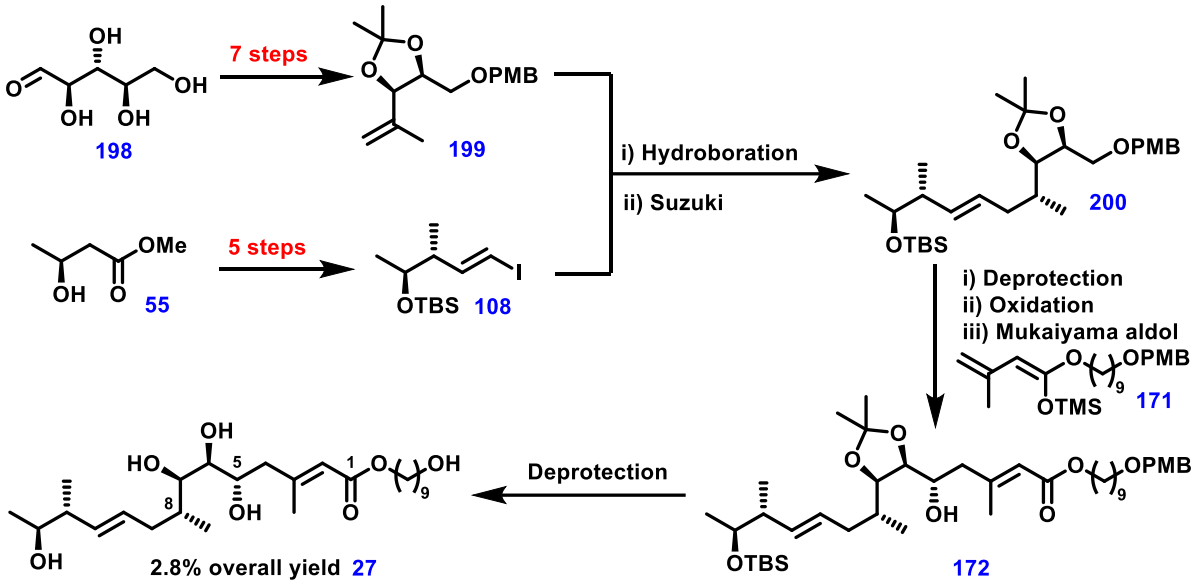
3.5 Synthetic efforts towards a mupirocin W4-OH analogue 196

To probe the specificities of MupW and MupZ further, work was undertaken to synthesise amine **196**, an analogue of desepoxy mupirocin W4-OH **27** bearing an amine group at C-5. If this substrate **196** was turned over by MupW/Z, a piperidine analogue of desepoxy PA-B **197** would be produced. Piperidine scaffolds are found in many biologically active compounds, so it would be interesting and useful to compare the structure activity relationship (SAR) of the pseudomonic acids with a piperidine analogue **197**, to determine its possible use as an antibiotic.



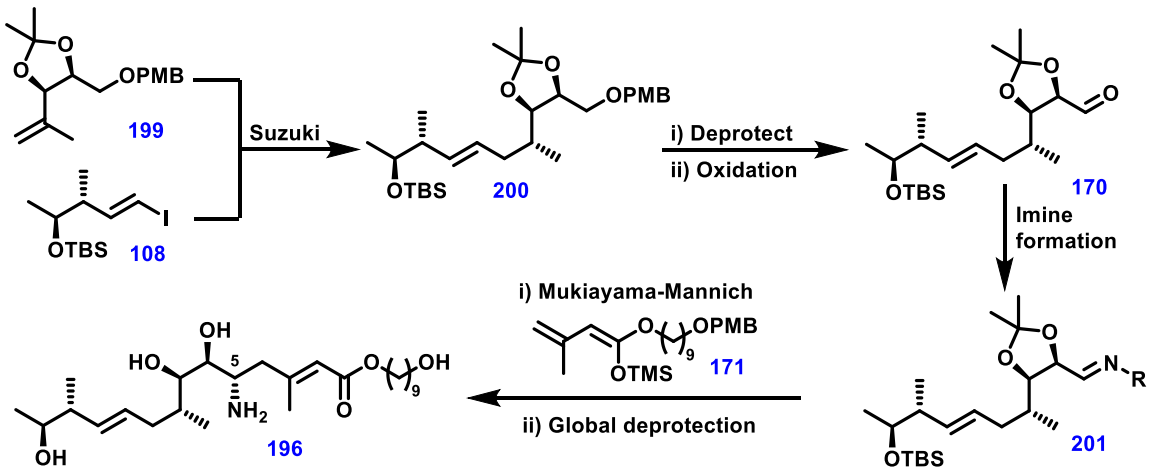
Scheme 125. The structures of amine analogue **196** and the desired piperidine **197**.

Due to the similarity in structure of amine **196** to desepoxy mupirocin W4-OH **27**, the beginning of the synthetic route utilised the chemistry developed by Dr Bakar in the synthesis of desepoxy mupirocin W4-OH **27** as shown in scheme 126.



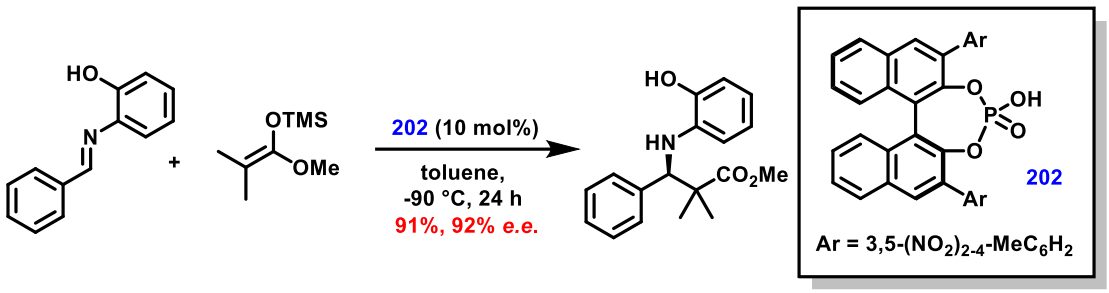
Scheme 126. Synthesis of mupirocin W4-OH **27** as carried out by Dr Bakar.

With respect to amine instillation, literature research gave two potential routes to substrate **196**. The first was a Mukaiyama-Mannich type reaction would directly install the fatty acid side chain and the amine simultaneously. This is proven to be an efficient way to install an amine group with high yields and high diastereoselectivity.[185-187]



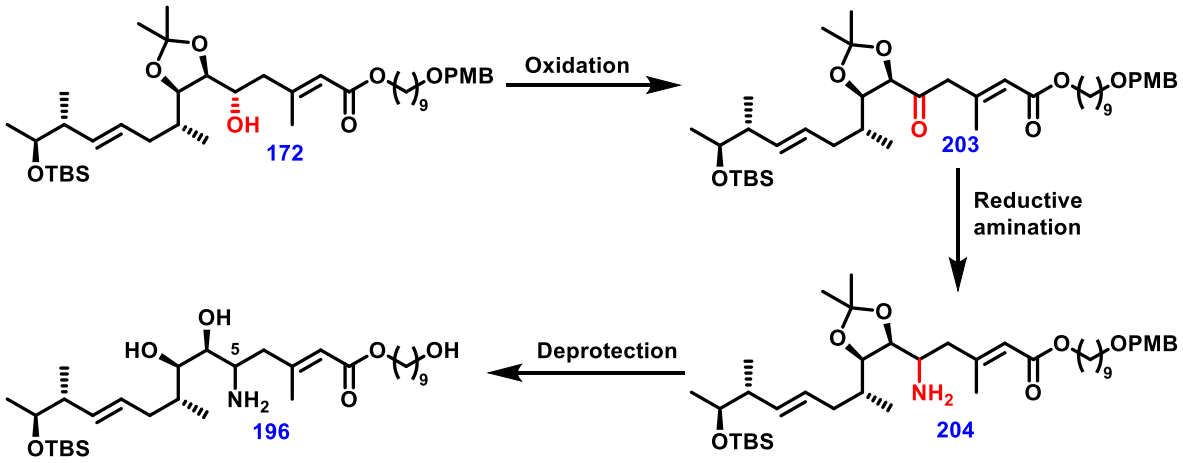
Scheme 127. A proposed method of installing the amine group at C-5.

Zhou *et al*. reported that with the development of a new BINOL derived chiral phosphoric acid catalyst **202**, β-amino carbonyl compounds could be synthesised with high yields and high *e.e*.[188]



Scheme 128. A novel catalyst developed by Zhou *et al.* for the use in Mukaiyama-Mannich reactions.[188]

The second potential route would include the Mukaiyama aldol reaction between aldehyde **170** and silyl dienol ether **171** as in the synthesis carried out by Dr Bakar (scheme 126), followed by an oxidation in order to enable the amine moiety to be installed via reductive amination as shown in scheme 127. Although this would likely give a mixture of diastereomers at the C-5 position, both substrates with natural and unnatural stereochemistry would be interesting to use in enzyme assays, especially as unnatural substrate **187** had been recognised and turned over by MupW and MupZ.

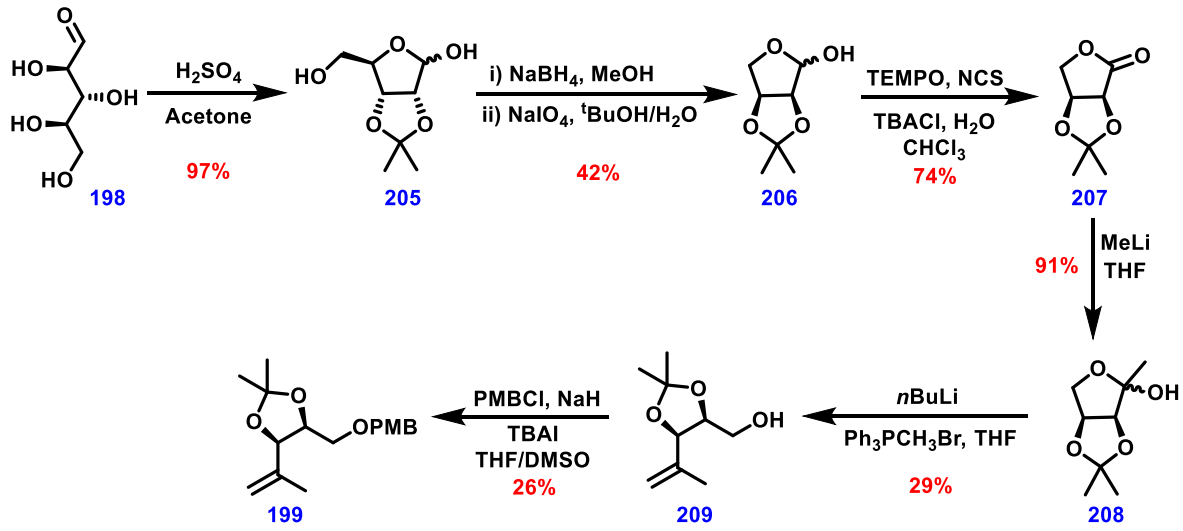


Scheme 129. A proposed synthesis for the installation of the amine at C-5.

3.5.1 Synthesis of core alkene 199

As the chemistry to alcohol **172** is known, it was decided to carry out the Mukaiyama aldol reaction to give **172**, followed by an oxidation to ketone **203** and reductive amination to give amine **204** as shown in scheme 129. With this in mind, the route to **172** began with the synthesis of the core fragment alkene **199**, following the approach developed by Dr Bakar in the synthesis of desepoxy mupirocin W4-OH **27**.

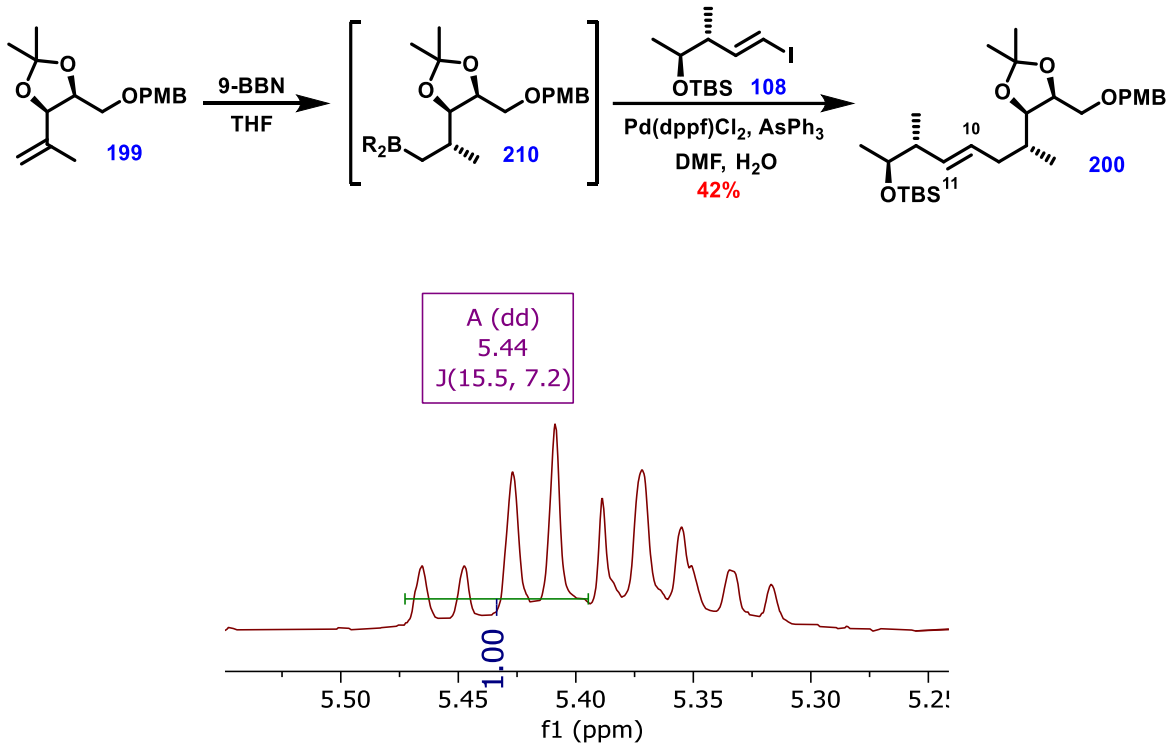
D-Ribose **198** was protected as the acetonide using acetone and H_2SO_4 to give lactol **205** in 85% yield (scheme 130). A one-pot reduction of **205** with $NaBH_4$ and periodate cleavage gave lactol **206** in 42% yield over two steps.



Scheme 130. Preparation of core fragment **199**.

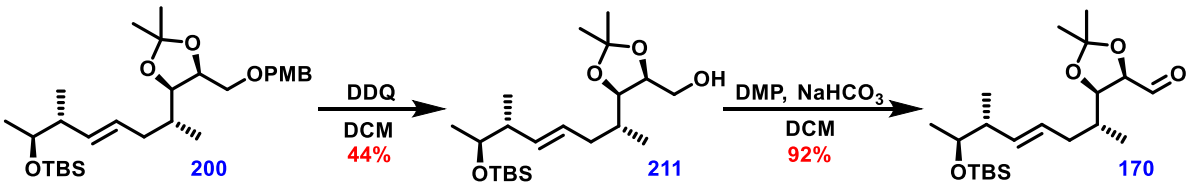
Lactol **206** was then oxidised to lactone **207** using mild TEMPO/NCS conditions[189] in the presence of TBACl, which acts as a phase transfer catalyst and allows the reaction to be carried out in a biphasic solvent system which in this case was $CHCl_3/H_2O$. Although the mechanism of TEMPO oxidation is the subject of debate, it is widely regarded to be a single electron transfer (SET) oxidant, while the NCS acts as the secondary oxidant.[189] Addition of MeLi to lactone **207** gave lactol **208**, which was then converted to alkene **209** in 29% yield via a Wittig reaction. Due to the incompatability of the free alcohol with the Suzuki conditions as discussed in chapter two (page 60) in the synthesis of thioester **29**, primary alcohol **209** was protected using PMBCl to give **199** in 26% yield.

The core fragment **199** was hydroborated using 9-BBN to give organoborane **210**, which was then reacted with vinyl iodide **108** under Suzuki conditions (scheme 131), generating alkene **200** in 42% yield as a single diastereomer with *E* alkene geometry. This was confirmed by ^{1}H NMR analysis of the signal assigned to 11-H, which had a coupling constant of 15.5 Hz (scheme 131).



Scheme 131. Suzuki coupling of **210** and **108** to give **200** with *E* alkene geometry exclusively. ^{1}H NMR spectrum showing the 11-H proton and its coupling constants.

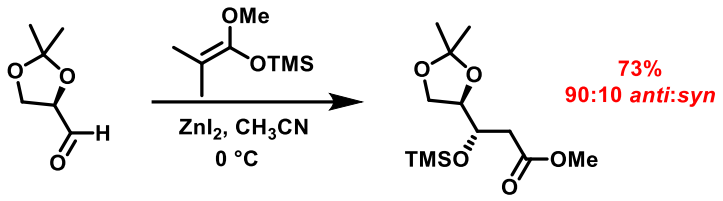
In order to complete the synthesis of aldehyde **170**, PMB ether **200** was deprotected using DDQ to give alcohol **211**, which was oxidised using DMP to aldehyde **170** in 40% yield over two steps (scheme 132).



Scheme 132. Synthesis of aldehyde **170** from PMB ether **200**.

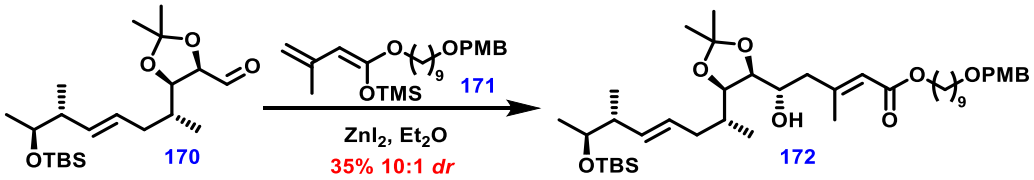
The Mukaiyama aldol reaction had to be approached differently than in the synthesis of substrate **161** (page 87, scheme 113), due to the sensitivity of the acetonide to strong Lewis acids. Kita *et al.* reported that high yields and stereoselectivity could be achieved when

acetonide protected glyceraldehyde derivatives were reacted with *O*-silylated ketene acetals in the presence of zinc and zirconium based Lewis acids, with the products retaining the acetonide protecting group (scheme 133).[182] When $ZrCl_4$ and ZnI_2 were used a *dr* of greater than 90% was observed, while $ZnBr_2$ and $ZnCl_2$ gave over 70% *dr*. Interestingly it was reported that with the widely used Lewis acids $TiCl_4$, $BF_3.OEt_2$ and $SnCl_4$, no satisfactory result was achieved.[182]



Scheme 133. The reaction of an *O*-silylated ketene acetal with an acetonide protected glyceraldehyde derivative as carried out by Kita *et al*.[190]

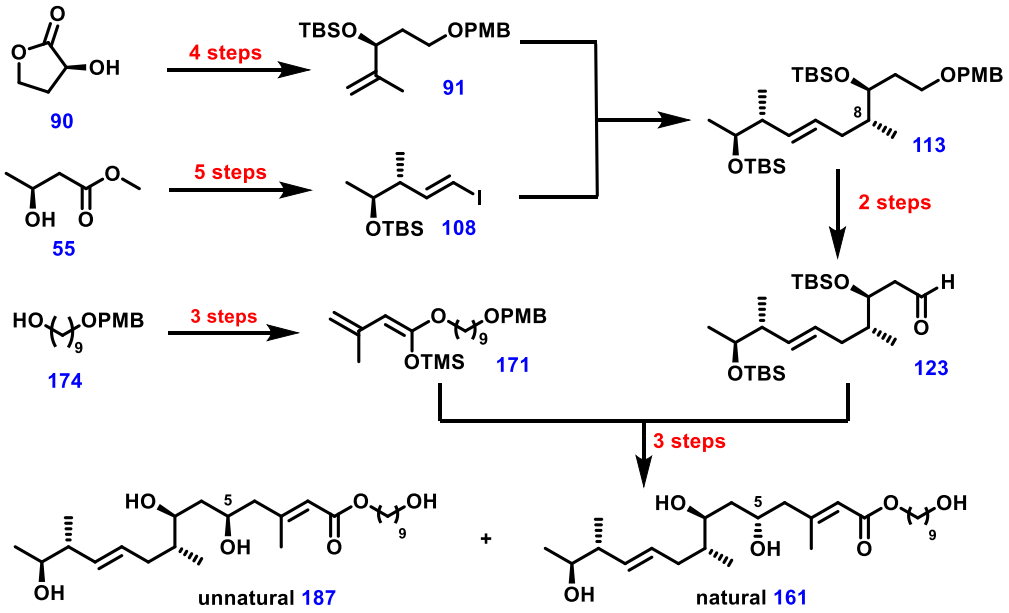
Dr Bakar achieved the best results when aldehyde **170** was reacted with TMS *O*-silylated ketene acetal **171** in the presence of ZnI_2 at 0 °C, which gave alcohol **172** with 90% *dr*. In my hands, none of the desired product **172** was isolated and due to time constraints, this reaction was not reattempted.



Scheme 134. The successful Mukaiyama aldol reaction of aldehyde **170** with silyl dienol ether **171** carried out by Dr Bakar.

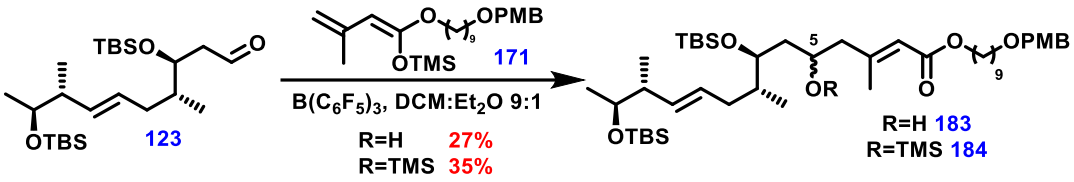
3.6 Conclusions and future work

The total synthesis of substrates **161** and **187** was achieved in 12 longest linear steps and 2.2% (natural) and 2.4% (unnatural) yield respectively. The key steps involved a Suzuki reaction which introduced the alkene with excellent control of *E*-geometry, a hydroboration which set the stereocentre at C-8. The synthetic route developed in the synthesis of thioester **29** (chapter two) was adapted in order to install the alcohol at C-5, utilising a key Mukaiyama aldol reaction.



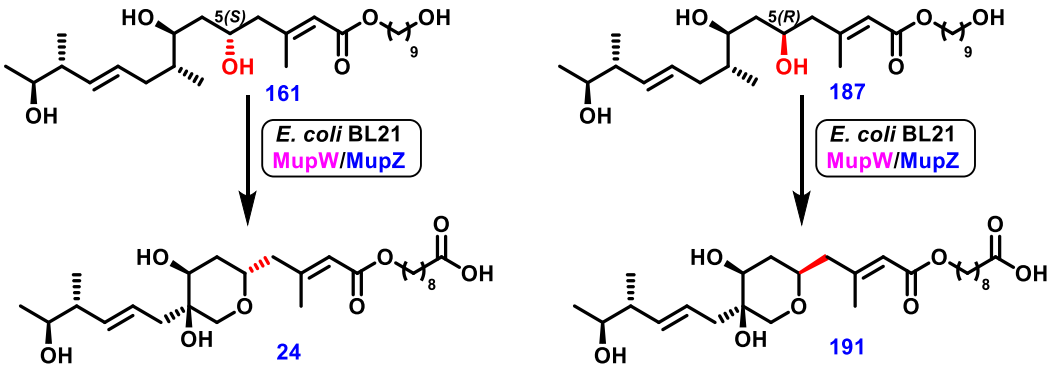
Scheme 135. Synthesis of **161** and **187**.

After a series of failed attempts at the VMAR, $B(C_6F_5)_3$ was used as the Lewis acid, which gave silyl ethers **183** and **184** as a mixture of diastereomers epimeric at C-5. This was serendipitous as the unnatural diastereomer proved to give interesting results in the biotransformation studies.



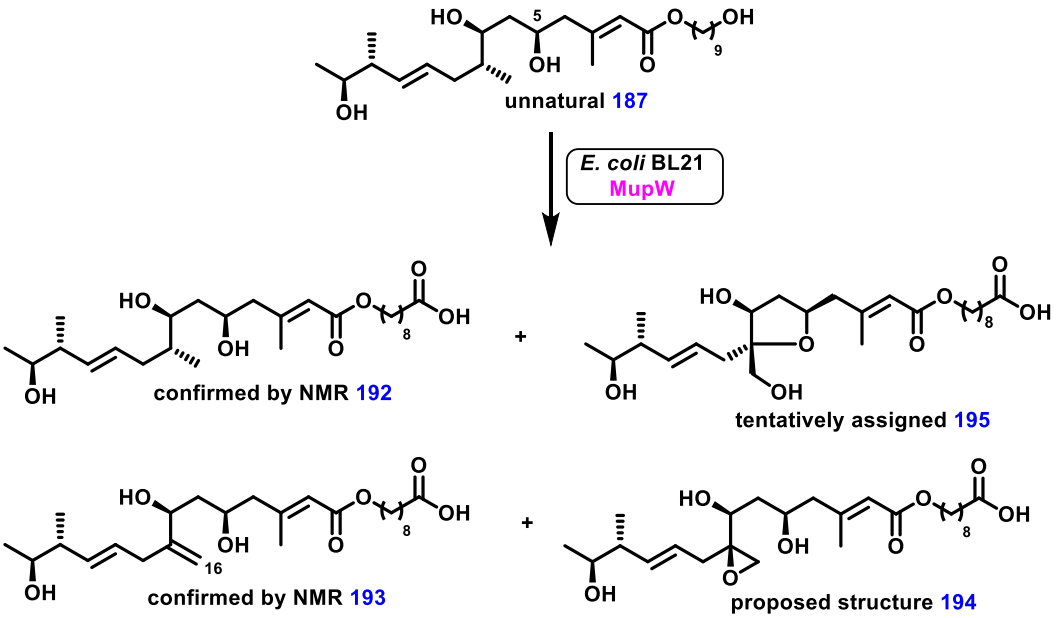
Scheme 136. Mukaiyama aldol reaction between **123** and **171**.

Both substrates **161** and **187** were recognised and turned over by MupW and MupZ to give the 6-membered THP ring products (scheme 137). Interestingly, HPLC analysis of natural substrate **161** showed this substrate could be cyclised before oxidation, which suggests that oxidation is not a prerequisite for cyclisation to occur. THP **24** was isolated by preparative HPLC and the structure elucidated by 1D and 2D NMR (figure 32), however an insufficient amount of THP **191** was isolated for full characterisation by NMR spectroscopy.



Scheme 137. THP products **24** and **191** produced by the incubation of alcohols **161** and **187** with MupW and MupZ.

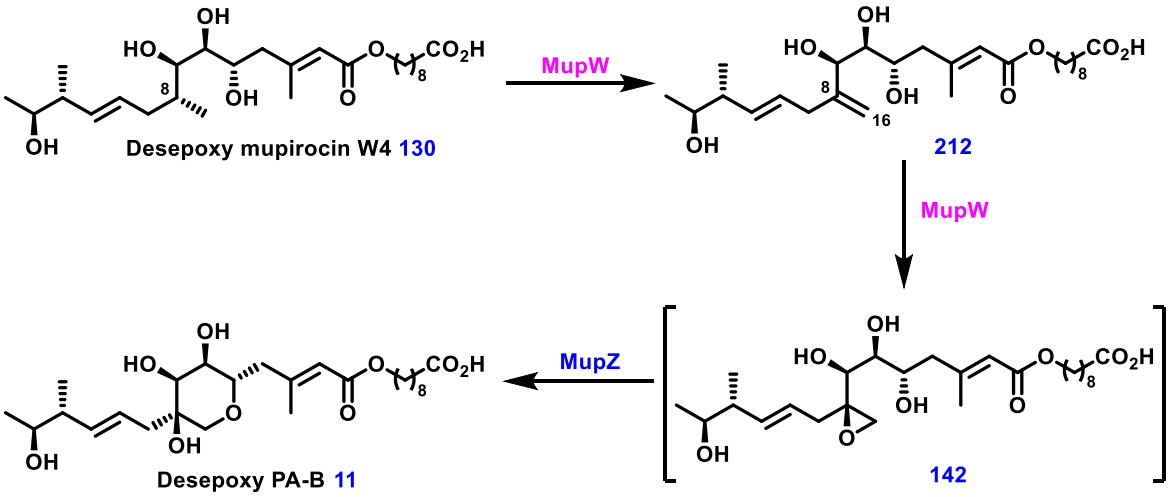
Bioassays with the unnatural substrate (5*R*) **187** were particularly interesting due to the intermediate compounds that were observed by HPLC. When this substrate **187** was incubated in *E. coli* expressing both MupW, a peak with a mass corresponding to 8,16-alkene **193** was observed. This was isolated by preparative HPLC and the structure confirmed by NMR spectroscopy.



Scheme 138. Incubation of unnatural substrate **187** with *E. coli* expressing MupW and MupZ and the observed metabolites.

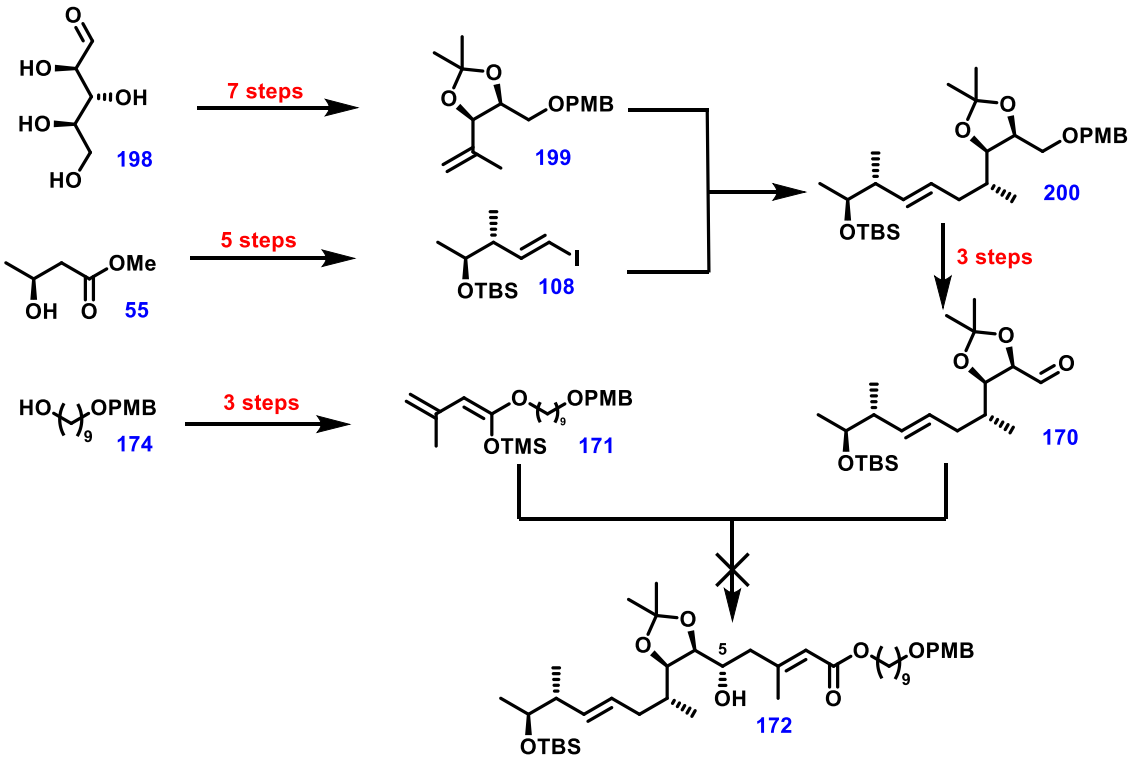
In addition, a novel peak in the HPLC trace with a mass that corresponds to the proposed epoxide intermediate **194** was observed. This bioassay was repeated on a larger scale and isolation of this peak attempted by HPLC, however this proposed intermediate was present in too small an amount to be able to determine its structure by NMR.

Alkene **193** isolated from feeding studies of unnatural substrate **187** with MupW in *E. coli* (scheme 138) was re-fed to *E. coli* expressing MupW, and the expected THF **195** was observed by HPLC-MS, indicating that alkene **193** is a true intermediate in the formation of THF **195**. From this result, we can infer that in PA-C biosynthesis the 8-methyl group is converted to the 8-16-alkene **212**, before epoxidation catalysed by MupW. In the presence of MupZ, this proposed epoxide intermediate undergoes ring closure to give desepoxy PA-B **11**, a precursor to PA-C **6**.



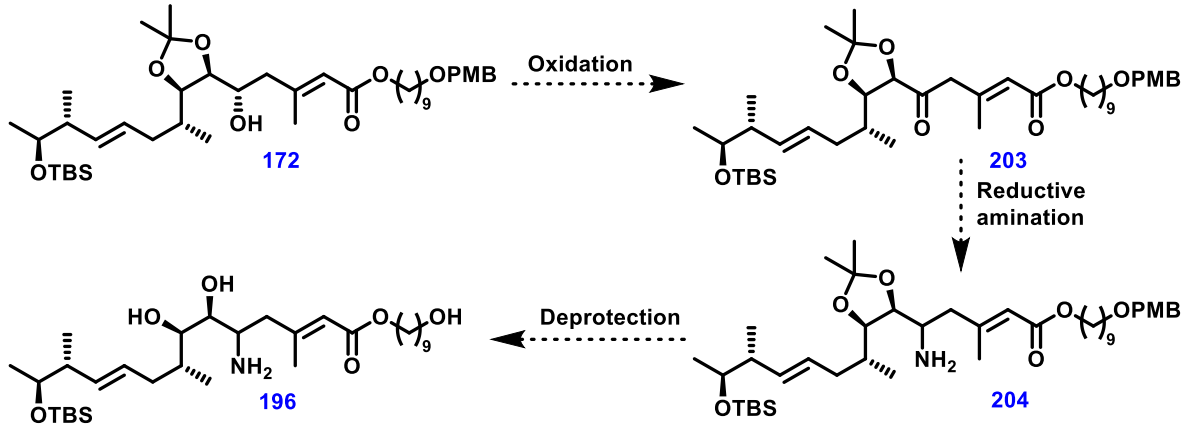
Scheme 139. The proposed mechanism for the conversion of desepoxy mupirocin W4 **130** to desepoxy PA-B **11**.

To probe the specificities of MupW and MupZ further, work was started towards the synthesis of amine **196**, an analogue of desepoxy mupirocin W4-OH **27**. By utilising the synthetic strategy employed by Dr Bakar in the total synthesis of desepoxy mupirocin W4-OH,[89] the synthesis of aldehyde **170** was achieved successfully, however in my hands the Mukaiyama aldol between aldehyde **170** and **171** was unsuccessful. Further work will be undertaken to complete this synthesis and install the amine moiety at C-5 by optimisation of the Mukaiyama aldol reaction between **170** and **171**.



Scheme 140. The starting steps of the synthesis of amine **196**.

Once conditions have been established for the synthesis of alcohol **172**, work can be undertaken to install the amine as shown in scheme 141.



Scheme 141. The final steps towards the synthesis of amine **196**.

Work is currently being undertaken to synthesise alkene **212** and alcohols **213** and **214** (figure 34), which will be used in biotransformation studies with MupW to probe the biosynthetic origin of the proposed epoxide intermediate **142**. In addition to this, work is ongoing to scale up the bioassay with tetraol **187** in *E. coli* expressing MupW to isolate the proposed epoxide **194** in order to verify our hypothesis.

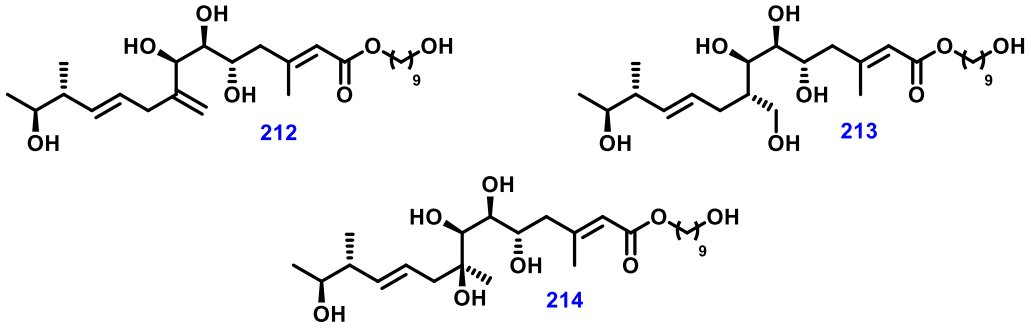


Figure 34. Alkene **212** and alcohols **213** and **214,** currently being synthesised for use in biotransformation studies.

In addition to amine **190**, a further substrate scope will be carried out including the des-7-hydroxy **215** and des-5-hydroxy **216** substrates. Tetraol **216** would be of particular interest as a non-turnover substrate due to the lack of hydroxyl group at C-5 preventing cyclisation. It is hoped that crystallisation studies of this compound could show the epoxide bound in the active site of MupW, giving further insight into this transformation.

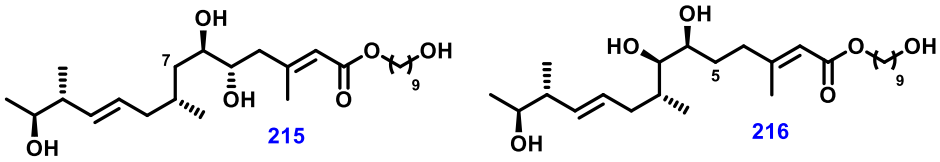


Figure 35. Tetraols **215** and **216** for use in a substrate scope of MupW and MupZ.

A long term aim for the MupW/MupZ work would be to develop a novel biocatalyst that can form ring systems from acyclic precursors by point mutations of the enzymes active sites. From this it would be possible to build up complex functionality in a molecule that would be otherwise difficult to achieve synthetically.

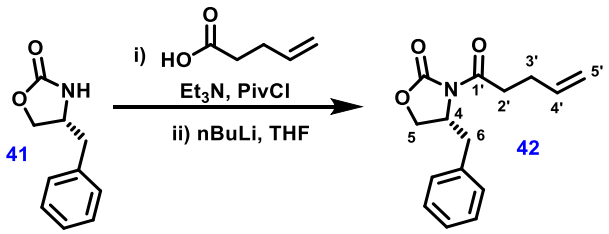
CHAPTER 4: Experimental

4. Experimental

General Experimental

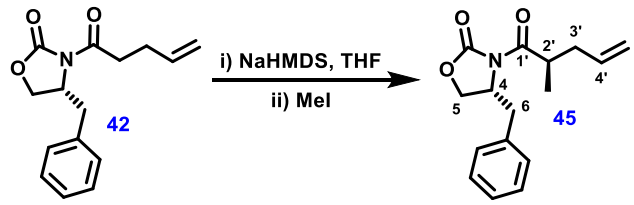
Commercially available compounds were used without further purification. Experiments which were air sensitive were carried out in flame-dried glassware under a positive pressure of nitrogen using standard syringe techniques. Anhydrous solvents dichloromethane, diethyl ether, tetrahydrofuran, and toluene were obtained by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering. Petroleum ether is of the 40-60 °C boiling point range. Phosphate buffer was purchased from Sigma Aldrich at pH 7.2 and 0.1 M concentration unless otherwise stated. Routine monitoring of reactions was performed using precoated Merck-Keiselgel 60 F_{254} aluminium backed TLC plates. The spots were visualised by UV_{254} light, or potassium permanganate. Flash column chromatography was performed using silica gel (40-63 micron, obtained by Sigma Aldrich) as the adsorbent and carried out according to the procedure outlined by Still *et al.*[191] Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer using either the neat compound or the compound dissolved in chloroform. Optical rotations were recorded using a Bellingham and Stanley ADP220 polarimeter, irradiating with the sodium D line (λ = 589 nm), and [α] values are quoted as in units 10^{-1} deg cm^2 g^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD unless stated otherwise. The spectra were recorded on a Bruker 400 MHz spectrometer unless otherwise stated. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constant (*J*) are in Hertz (Hz). HSQC NMR were routinely used to definitively assign the signals of ^{13}C NMR spectra. Electron ionisation (EI) was recorded on a VG analytical Autospec mass spectrometer. Electrospray ionisation (ESI) was recorded on a Bruker Daltonics Apex 4e 7.0T FT-MS mass spectrometer. Methane was the ionised gas used for the chemical ionisation. Unless stated, data for all known compounds are in agreement with published data.

(*R*)-4-Benzyl-3-(pent-4-enoyl)oxazolidin-2-one 42



4-Pentenoic acid (1.00 mL, 10.2 mmol) in anhydrous THF (5 mL) was cooled to -78 °C under N_2. Triethylamine (1.81 mL, 13.2 mmol) was added and then pivaloyl chloride (1.25 mL, 10.2 mmol) was added dropwise. The reaction mixture was stirred at -78 °C for 30 mins, then warmed to RT for 2 h before being cooled again to -78 °C. In a separate flask, Evans' auxiliary **41** (1.64 g, 10.2 mmol) in anhydrous THF (10 mL) was cooled to -78 °C under N_2. nBuLi (1.6 M in hexanes, 6.45 mL, 10.2 mmol) was added slowly at -78 °C and the mixture stirred for 30 mins at RT. The anion was slowly transferred to the mixed anhydride and stirred at RT for 16 h. The reaction mixture was quenched with $NaHCO_3$ (20 mL), extracted with EtOAc (3 x 20 mL) and the combined organic extracts dried ($MgSO_4$). The solvent was removed *in vacuo* to give a pale yellow oil which was purified by column chromatography (10%-20% EtOAc in petrol) to give **42** as a colourless oil (2.10 g, 81%); δ_H (400 MHz, $CDCl_3$) 7.25 (3 H, m, Ar-H), 7.15 (2 H, m, Ar-H), 5.81 (1 H, m, 4'-H), 5.00 (2 H, m, 5'-H_2), 4.62 (1 H, m, 5-H*H*), 4.12 (1 H, m, 5-*H*H), 4.05 (1 H, m, 4-H), 3.25 (1 H, dd, *J* 13.0, 3.0, 3'-*H*H), 2.97 (2 H, m, 6-H_2), 2.71 (1 H, m, 3'-H*H*), 2.40 (2 H, m, 2'-H_2); δ_C (100 MHz, $CDCl_3$) 172.5 (C-2), 153.4 (C-1'), 136.7 (C-4'), 135.2 (Ar-C), 129.4 (Ar-C), 129.0 (Ar-C), 127.3 (Ar-C), 115.7 (C-5'), 66.2 (C-5), 55.2 (C-4), 38.1 (C-3'), 37.9 (C-6), 28.2 (C-2'). Data in accordance with the literature.[192]

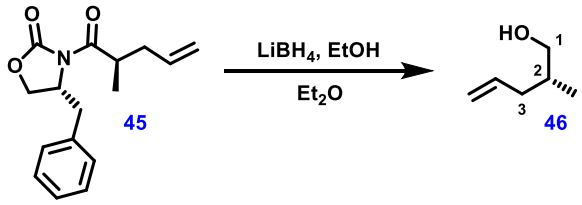
(*R*)-4-Benzyl-3-((*R*)-2-methylpent-4-enoyl)oxazolidin-2-one 45



A solution of the alkene **42** (0.20 g, 1.18 mmol), in anhydrous THF (4 mL) under N_2 was cooled to -78 °C and NaHMDS (1 M in hexanes, 1.77 mL, 1.77 mmol) was added dropwise and stirred for 1 h, followed by the dropwise addition of MeI (0.21 mL, 4.72 mmol) at -78 °C. After

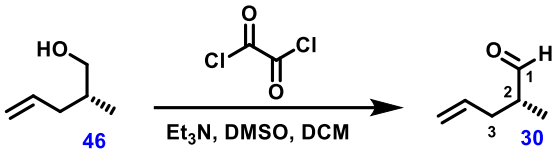
stirring for 1 h, the reaction was quenched with sat. aq. NH_4Cl (10 mL) and extracted with EtOAc (3 x 15 mL). The combined organics were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale orange oil. This was purified by column chromatography (20% EtOAc in petrol) to give **45** as a colourless oil (0.23 g, 88%); $[\alpha]_D^{24}$ -2.0 (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.22 (3 H, m, Ar-H), 7.11 (2 H, m, Ar-H), 5.66 (1 H, m, 4'-H), 4.93 (2 H, m, 5'-H_2), 4.53 (1 H, m, 5-*H*H), 4.08 (1 H, m, 5-H*H*), 4.06 (1 H, m, 4-H), 3.71 (1 H, m, 2'-H), 3.15 (1 H, dd, *J* 13.0, 3.0, 3'-*H*H), 2.66 (1 H, m, 3'-H*H*), 2.37 (1 H, m, 6-H*H*), 2.08 (1 H, m, 6-*H*H), 1.13 (3 H, d, *J* 7.0, 2'-CH_3); δ_C (100 MHz, $CDCl_3$) 136.3 (C-4'), 135.2 (Ar-C), 129.7 (Ar-C), 128.3 (Ar-C), 127.3 (Ar-C) 117.5 (C-5'), 69.0 (C-4), 65.7 (C-5), 38.9 (C-2'), 38.6 (C-3'), 38.1 (C-6), 17.6 (2'-CH_3). Data in accordance with the literature.[192] No optical rotation recorded in literature.

(*R*)-2-Methylpent-4-en-1-ol 46



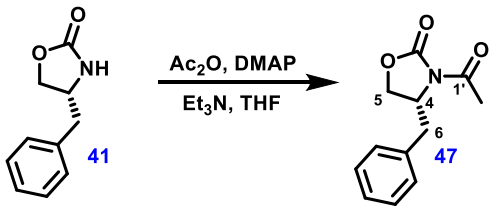
Alkene **45** (0.20 g, 0.73 mmol) was dissolved in anhydrous Et_2O (5 mL) and cooled to 0 °C under N_2. To this solution was added EtOH (0.06 mL, 1.09 mmol) followed by the dropwise addition of $LiBH_4$ (2 M in THF, 0.51 mL, 1.02 mmol). The solution was stirred for 1 h at 0 °C and then 3 h at RT. The reaction was quenched with NaOH (1 M, 5 mL) and extracted with EtOAc (3 x 15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a colourless oil, which was purified by column chromatography (40% EtOAc in petrol) to give alcohol **46** as a colourless oil (52 mg, 71%); δ_H (400 MHz, $CDCl_3$) 5.74 (1 H, m, 4-H), 4.96 (2 H, m, 5-H_2), 3.42 (2 H, m, 1-H_2), 2.10 (1 H, m, 3-*H*H), 1.87 (1 H, m, 3-*H*H), 1.68 (1 H, m, 2-H), 0.85 (3 H, d, *J* 7.0, 2-CH_3); δ_C (100 MHz, $CDCl_3$) 137.5 (C-4), 116.7 (C-5), 68.3 (C-1), 37.8 (C-3), 35.3 (C-2), 20.4 (2-CH_3). Data in accordance with the literature.[193]

(*R*)-2-Methylpent-4-enal 30



A solution of oxalyl chloride (0.12 mL, 1.43 mmol) in anhydrous DCM (5 mL) was cooled to -78 °C and DMSO (0.20 mL, 2.86 mmol) added under N_2. After stirring for 15 mins a solution of alcohol **46** (130 mg, 1.31 mmol) in anhydrous DCM (5 mL) and Et_3N (0.91 mL, 6.50 mmol) were added successively. The reaction was stirred at RT for 3 h before being quenched with H_2O (10 mL) and extracted with DCM (3 x 15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give aldehyde **30** as an orange oil (80 mg, 63%); δ_H (400 MHz, $CDCl_3$) 9.51 (1 H, s, 1-H), 5.61 (1 H, m, 4-H), 4.97 (2 H, m, 5-H_2), 2.31 (2 H, m, 3-H_2), 2.01 (1 H, m, 2-H), 0.97 (3 H, d, *J* 7.0, CH_3); δ_c (100 MHz, $CDCl_3$) 134.9 (C-4), 117.3(C-5), 46.2 (C-1), 41.0 (C-3), 34.7 (C-2), 11.5 (CH_3). Data in accordance with the literature.[194]

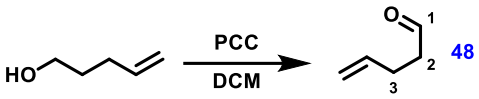
(*R*)-3-Acetyl-4-benzyloxazolidin-2-one 47



To Evans' auxiliary **41** (500 mg, 3.06 mmol) in anhydrous THF (10 mL) was added DMAP (7.3 mg, 0.06 mmol) and Et_3N (0.42 mL, 3.06 mmol) under N_2. The reaction mixture was kept at between 0 °C and 10 °C while Ac_2O (0.57 mL, 6.12 mmol) was added dropwise over 5 min. The reaction mixture was stirred at RT for 15 h. After this time, TLC analysis showed the reaction to have not reached completion so DMAP (7.3 mg, 0.06 mmol) and Ac_2O (0.57 mL, 6.12 mmol) were added. The reaction was stirred for 1.5 h before the volatiles were removed *in vacuo* and the resulting oil extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with water (10 mL), followed by brine (15 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give **47** as a colourless solid (630 mg, 94%); δ_H (400 MHz, $CDCl_3$) 7.36 (3 H, m, Ar-H), 7.24 (2 H, m, Ar-H), 4.69 (1 H, m, 4-H), 4.20 (2 H, m, 6-H_2), 3.32 (1 H, dd, *J* 13.0, 3.0, 5-*H*H), 2.80 (1 H, dd, *J* 13.0, 10.0, 5-*H*H), 2.59 (3 H, s, 2'-CH_3); δ_c (100 MHz,

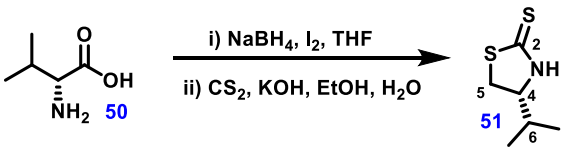
$CDCl_3$) 170.3 (C-1'), 153.6 (Ar-H), 135.2 (C-2), 129.4 (Ar-C), 129.0 (Ar-C), 127.4 (Ar-C), 66.0 (C-6), 55.0 (C-4), 37.9 (C-5), 23.8 (C-2'). Data in accordance with the literature.[195]

Pent-4-enal 48



PCC (1.88 g, 8.71 mmol) was suspended in anhydrous DCM (13 mL) to which 4-penten-1-ol (0.59 mL, 5.81 mmol) was added under N_2 and the reaction stirred for 3 h at RT. The reaction mixture was filtered through silica and celite (3:1) and the solvent removed *in vacuo* to give aldehyde **48** as a colourless oil (345 mg, 71%); δ_H (400 MHz, $CDCl_3$) 9.71 (1 H, s, CHO), 5.76 (1 H, m, 4-H), 4.98 (2 H, m, 5-H_2), 2.48 (2 H, m, 2-H_2), 2.32 (2 H, m, 3-H_2); δ_C (100 MHz, $CDCl_3$) 201.9 (C-1), 136.4 (C-4), 115.7 (C-5), 42.8 (C-2), 26.1 (C-3). Data in accordance with the literature.[196]

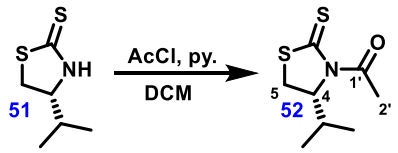
(*R*)-4-Isopropylthiazolidine-2-thione 51



To a solution of D-valine **50** (6.00 g, 51.1 mmol) in anhydrous THF (165 mL) at 0 °C was added $NaBH_4$ (4.54 g, 107 mmol) in one portion. The resulting mixture was stirred for 5 min before a solution of iodine (12.7 g, 51.1 mmol) in anhydrous THF (15 mL) was added slowly dropwise over 20 min. The reaction mixture was warmed to RT and stirred until no more effervescence was observed, then heated at reflux for 24 h. After this time the reaction was cooled to RT and MeOH (20 mL) was added dropwise until the reaction mixture became clear. Volatiles were removed *in vacuo* and the resultant white paste dissolved in an aqueous solution of KOH (3 M). The solution was stirred for 4 h before being extracted with DCM (3 x 40 mL). The combined organic extracts were washed with brine (10 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give D-valinol; lit.$[\alpha]_D^{25}$=-17.0; $[\alpha]_D^{24}$=-17.0 (*c* 3, EtOH). To a solution of D-valinol (6.70 g, 64.9 mmol) in EtOH (20 mL) was added CS_2 (10.2 mL, 169 mmol). A solution of KOH (2.25 M, 77.9 mL, 175 mmol) in a 1:1 mixture of EtOH (35 mL) and H_2O (35 mL) was added slowly over 20 mins. The reaction mixture was heated at reflux for 72 h. After

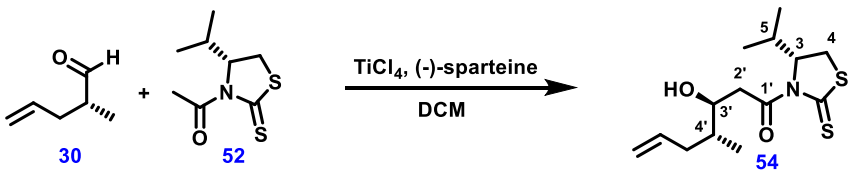
this time, the reaction was cooled to RT and the volatiles removed *in vacuo*. The resulting suspension was acidified with HCl (0.5 M, *ca*. 300 mL) and extracted with DCM (3 x 150 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give **51** as a colourless solid (8.20 g, 78%); lit.$[\alpha]_D^{25}$=+32.7; $[\alpha]_D^{24}$=+32.2 (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.48 (1 H, br s, NH), 4.04 (1 H, td, *J* 8.0, 6.5, 4-H), 3.51 (1 H, dd, *J* 11.0, 8.0, 5-*H*H), 3.33 (1 H, dd, *J* 8.0, 11.0, 5-H*H*), 2.00-1.90 (1 H, m, 6-H), 1.05 (3 H, d, *J* 6.5, CH_3), 1.01 (3 H, d, *J* 6.5, CH_3); δ_C (100 MHz, $CDCl_3$) 201.2 (C-2), 69.7 (C-4), 36.1 (C-5), 32.0 (C-6), 18.7 (CH_3), 18.2 (CH_3). Data in accordance with the literature.[198]

(*R*)-1-(4-Isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one 52



Auxiliary **51** (8.20 g, 50.8 mmol) was dissolved in anhydrous DCM (200 mL) and AcCl (5.34 mL, 76.3 mmol) added under N_2 at RT. Pyridine (6.13 mL, 76.3 mmol) was added dropwise and the reaction mixture stirred at RT for 3 h, then concentrated *in vacuo*. The resulting residue was filtered and the solvent removed *in vacuo* to give an orange oil which was purified by column chromatography (30% EtOAc in hexanes) to give **52** as an orange oil (8.75 g, 85%); lit.$[\alpha]_D^{25}$=-450; $[\alpha]_D^{24}$=-442 (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.15 (1 H, ddd, *J* 8.0, 6.0, 1.0, 4-H), 3.50 (1 H, dd, *J* 11.5, 8.0, 5-*H*H), 3.02 (1 H, dd, *J* 11.5, 1.0, 5-*H*H), 2.77 (3 H, s, 2'-H_3), 2.36 (1 H, m, 6-H), 1.05 (3 H, d, *J* 7.0, CH_3), 0.97 (3 H, d, *J* 7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 203.6 (C-2), 171.1 (C-1'), 71.7 (C-4), 31.2 (C-6), 30.9 (C-5), 27.4 (C-2'), 19.5 (CH_3), 18.2 (CH_3). Data in accordance with the literature.[198]

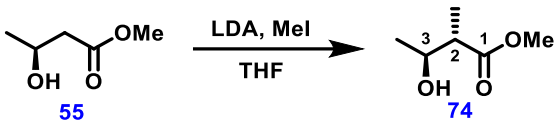
(3*S*,4*R*)-3-Hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)-4-methylhept-6-en-1-one 54



Auxiliary **52** (3.10 g, 15.3 mmol) was dissolved in anhydrous DCM (75 mL) under N_2 and $TiCl_4$ (3.35 mL, 30.6 mmol) was added dropwise at 0 °C. The reaction mixture was stirred for 10

mins, after which time the solution was cooled to – 40 °C and (-)-sparteine (1.93 mL, 15.3 mmol) was added. After stirring for 1 h, the reaction mixture was cooled to – 78 °C and aldehyde **30** (0.50 g, 5.09 mmol) in anhydrous DCM (5 mL) was added. The reaction mixture was stirred for a further 1.5 h before being quenched pH 7 buffer (20 mL) and extracted with DCM (3 x 40 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil which was purified by column chromatography (15% EtOAc in petrol) to give **54** as an orange oil (750 mg, 51%); $[\alpha]_D^{24}$ -330 (*c* 0.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.81 (1 H, m, 6'-H), 5.16 (1 H, ddd, *J* 7.5, 6.0, 1.0, 3-H), 5.03 (2 H, m, 7'-H_2), 3.99 (1 H, ddd, *J* 10.0, 6.0, 2.0, 3'-H), 3.61 (1 H, dd, *J* 18.0, 2.0, 2'-H*H*), 3.53 (1 H, dd, *J* 11.5, 7.5, 4-H*H*), 3.20 (1 H, m, 2'-H*H*), 3.05 (1 H, dd, *J* 11.5, 1.0, 4-H*H*), 2.36 (1 H, m, 5-H), 2.31 (1 H, m, 5'-H*H*), 1.97 (1 H, m, 4'-H), 1.73 (1 H, m, 5'-H*H*), 1.08 (3 H, d, *J* 7.0, 4'-CH_3), 0.99 (3 H, d, *J* 7.0, 5-CH_3), 0.91 (3 H, d, *J* 7.0, 5-CH_3); δ_C (100 MHz, $CDCl_3$) 203.1 (C-1'), 173.7 (C-1), 137.0 (C-6'), 116.3 (C-7'), 71.5 (C-3), 71.4 (C-3'), 42.5 (C-2'), 38.0 (C-5'), 37.0 (C-5), 37.0 (C-4'), 30.6 (C-4), 19.1 (4'-CH_3), 17.8 (5-CH_3), 15.2 (5-CH_3). Data in accordance with the literature.[117] Literature $[\alpha]_D^{24}$ -330 (*c* 0.4, $CHCl_3$).

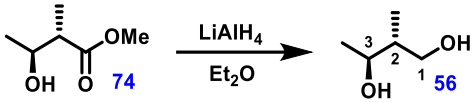
Methyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate 74



DIPA (3.77 mL, 26.7 mmol) was dissolved in anhydrous THF (20 mL) and cooled to -78 °C under N_2. nBuLi (16.7 mL, 26.7 mmol, 1.61 M in hexanes) was added dropwise and the reaction mixture stirred for 1 h. A solution of ester **55** (1.40 mL, 12.8 mmol) in anhydrous THF (10 mL) was added dropwise. The reaction mixture was stirred at RT for 20 mins, after which time it was cooled to -78 °C. MeI (0.96 mL, 15.4 mmol) was added dropwise and the reaction mixture stirred at 0 °C for 3 h after which time the reaction was quenched with HCl (6 M, 10 mL). The reaction was extracted with Et_2O (3 x 30 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (5% EtOAc in petrol) to give ester **74** as a colourless oil (1.20 g, 82%); δ_H (400 MHz, $CDCl_3$) 3.87 (1 H, p, *J* 7.0, 3-H), 3.69 (3 H, s, OCH_3), 2.44 (1 H, p, *J* 7.0, 2-H), 1.20 (3 H, d, *J* 7.0, 4-H_3), 1.15 (3-H, d, *J* 7.0, 2-CH_3); δ_C (100 MHz, $CDCl_3$) 176.3 (C-1), 63.4

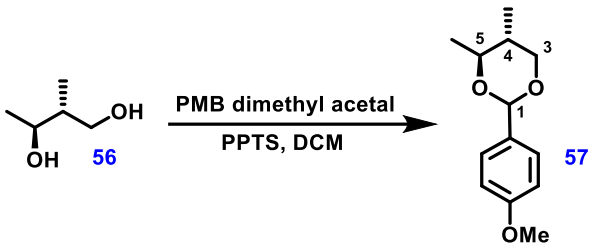
(C-3), 51.7 (OCH_3), 46.9 (C-2), 20.7 (2-CH_3), 14.0 (C-4). Data in accordance with the literature.[201]

(2*R*,3*S*)-2-Methylbutane-1,3-diol 56



$LiAlH_4$ (302 mg, 7.94 mmol) was suspended in anhydrous Et_2O (12 mL) and cooled to 0 °C under N_2. Ester **74** (500 mg, 3.78 mmol) in anhydrous Et_2O (1 mL) was added dropwise and the reaction mixture stirred for 1 h. The reaction was quenched by addition of H_2O (0.3 mL) followed by NaOH (1 M, 0.5 mL). The slurry was dried ($MgSO_4$) and filtered through a plug of celite. The solvent was removed *in vacuo* to give diol **56** as a pale yellow oil (381 mg, 97%). δ_H (400 MHz, $CDCl_3$) 3.72 (2 H, m, 1-H_2), 3.62 (1 H, m, 3-H), 1.67 (1 H, m, 2-H), 1.24 (3 H, d, *J* 7.0, 2-CH_3), 0.86 (3 H, d, *J* 7.0, 4-H_3); δ_C (100 MHz, $CDCl_3$) 73.7 (C-1), 68.2 (C-3), 41.8 (C-2), 22.1 (2-CH_3), 13.7 (C-4). Data in accordance with the literature.[202]

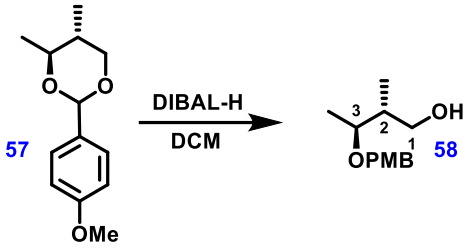
(4*S*,5*R*)-2-(4-Methoxyphenyl)-4,5-dimethyl-1,3-dioxane 57



To diol **56** (200 mg, 1.92 mmol) dissolved in anhydrous DCM (5 mL) was added *p*-methoxybenzaldehyde dimethyl acetal (419 mg, 2.30 mmol) and PPTS (95 mg, 0.38 mmol) under N_2. The reaction mixture was stirred at RT for 5 h, after which time the reaction was quenched with sat. aq. $NaHCO_3$ (5 mL). The reaction mixture was extracted with DCM (3 x 10 mL), the combined organic extracts dried ($MgSO_4$) and the solvent removed *in vacuo*. The resulting orange oil was purified by column chromatography (5% EtOAc in petrol) to give **57** as a colourless oil (392 mg, 92%); δ_H (400 MHz, $CDCl_3$) 7.36 (2 H, d, *J* 9.0, Ar-H), 6.80 (2 H, d, *J* 9.0, Ar-H), 5.38 (1 H, s, 1-H), 4.03 (1 H, m, 3-*H*H), 3.73 (3 H, s, OMe), 3.49 (1 H, m, 5-H), 3.41 (1 H, m, 3-H*H*), 1.69 (1 H, m, 4-H), 1.23 (3 H, d, *J* 7.0, 4-CH_3), 0.72 (3 H, d, *J* 7.0, 6-H_3); δ_C (100 MHz, $CDCl_3$) 159.9 (Ar-C), 131.3 (Ar-C), 127.5 (Ar-C), 113.6 (Ar-C), 102.0 (C-1), 79.5 (C-5), 72.9

(C-3), 55.3 (OMe), 35.9 (C-4), 19.3 (4-CH_3), 12.5 (C-6). Data in accordance with the literature.[203]

(2*R*,3*S*)-3-((4-Methoxybenzyl)oxy)-2-methylbutan-1-ol 58



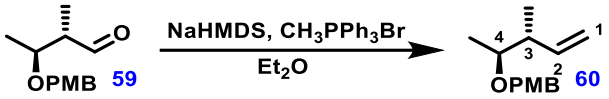
Acetal **57** (250 mg, 1.13 mmol) was dissolved in anhydrous DCM (6 mL) and cooled to – 50 °C under N_2. DIBAL-H (1 M in DCM, 5.65 mL, 5.65 mmol) was added dropwise and the reaction mixture stirred for 1.5 h at 0 °C, after which time the reaction was quenched by addition of MeOH (2 mL) and Rochelle's salt (15 mL). The reaction mixture was stirred vigorously overnight before being extracted with Et_2O (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a colourless oil. This was purified by column chromatography (1% EtOAc in petrol) to give **58** as a pale yellow oil (232 mg, 92%); δ_H (400 MHz, $CDCl_3$) 7.26 (2 H, d, *J* 8.0, Ar-H), 6.87 (2 H, d, *J* 8.0, Ar-H), 4.51-4.28 (2 H, m, OCH_2), 3.73 (3 H, s, OMe), 3.57-3.49 (2 H, m, 1-H_2), 3.39 (1 H, m, 3-H), 1.70 (1 H, m, 2-H), 1.17 (3 H, d, *J* 8.0, 2-CH_3), 0.82 (3 H, d, *J* 8.0, 4-H_3); δ_C (100 MHz, $CDCl_3$) 159.3 (Ar-C), 130.3 (Ar-C), 129.4 (Ar-C), 113.9 (Ar-C), 80.2 (C-3), 70.6 (OCH_2), 67.2 (C-1), 55.3 (OMe), 41.2 (C-2), 17.4 (2-CH_3), 14.1 (C-4). Data in accordance with the literature.[203]

(2*S*,3*S*)-3-((4-Methoxybenzyl)oxy)-2-methylbutanal 59

To alcohol **58** (250 mg, 1.11 mmol) in anhydrous DCM (5 mL) was added TEMPO (70 mg, 0.45 mmol) and BAIB (538 mg, 1.67 mmol) under N_2. The reaction mixture was stirred for 3 h at RT, then quenched with sat. aq. $Na_2S_2O_3$ (5 mL) and extracted with Et_2O (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (3% EtOAc in petrol) to give aldehyde **59** as a colourless oil (221 mg, 90%); δ_H (400 MHz, $CDCl_3$) 9.64 (1 H, s, 1-H), 7.16 (2 H, d, *J* 8.0, Ar-H), 6.81 (2 H, d, *J* 8.0, Ar-H), 4.47-4.32 (2 H, m, OCH_2), 3.72 (3 H, s, OMe), 3.72

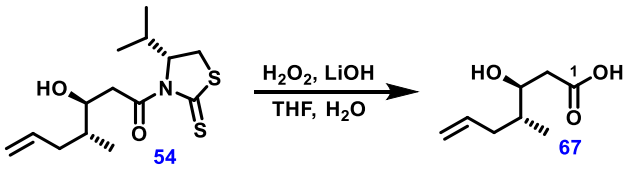
(1 H, m, 3-H), 2.47 (1 H, m, 2-H), 1.17 (3 H, d, *J* 7.0, 2-CH_3), 0.87 (3 H, d, *J* 7.0, 4-H_3); δ_C (100 MHz, $CDCl_3$) 204.6 (C-1), 159.3 (Ar-C), 130.3 (Ar-C), 129.3 (Ar-C), 113.9 (Ar-C), 75.0 (OMe), 74.4 (OCH_2), 55.3 (C-3), 51.2 (C-2), 16.9 (2-CH_3), 10.2 (C-4). Data in accordance with the literature.[204]

(2*S*,3*S*)-3-((4-Methoxybenzyl)oxy)-3-methylpent-1-ene 60



CH_3PPh_3Br (475 mg, 1.33 mmol) was suspended in anhydrous Et_2O (5 mL) and cooled to -78 °C under N_2. NaHMDS (1 M in Et_2O, 1.3 mL, 1.30 mmol) was added and stirred for 1 h. Aldehyde **59** (1.11 mmol) in anhydrous Et_2O (5 mL) was added dropwise and the reaction stirred for 16 h at RT. The reaction was quenched with sat. aq. NH_4Cl (5 mL) and extracted with Et_2O (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (1% EtOAc in petrol) to give **60** as a colourless oil (234 mg, 80%); δ_H (400 MHz, $CDCl_3$) 7.16 (2 H, d, *J* 8.0, Ar-H), 6.74 (2 H, d, *J* 8.0, Ar-H), 5.68 (1 H, m, 2-H), 4.91- 4.87 (2 H, m, 1-H_2), 4.37-4.26 (2 H, m, OCH_2), 3.66 (3 H, s, OMe), 3.29 (1 H, m, 4-H), 2.25 (1 H, m, 3-H), 0.97 (3 H, d, *J* 7.0, 3-CH_3), 0.87 (3 H, d, *J* 7.0, 5-H_3); δ_C (100 MHz, $CDCl_3$) 159.1 (Ar-C), 141.2 (C-2), 131.2 (Ar-C), 129.2 (Ar-C), 114.3 (Ar-C), 77.8 (OCH_2), 70.3 (OMe), 65.9 (C-4), 55.3 (C-3) 42.5 (C-1), 16.1 (C-5), 14.7 (3-CH_3). Data in accordance with the literature.[204-205]

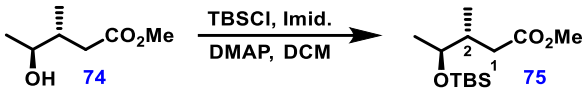
(3*S*,4*R*)-3-Hydroxy-4-methylhept-6-enoic acid 67



To a stirred solution of **54** (200 mg, 0.66 mmol) in THF/H_2O (6.5 mL/1.5 mL) was added H_2O_2 (30% w:v, 1.8 mL, 5.31 mmol) and aq. LiOH (1 M, 2.7 mL) at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 24 h after which time sat. aq. $Na_2S_2O_4$ (*ca.* 5 mL) was added and the reaction mixture extracted with Et_2O (3 x 15 mL). The aqueous layer was then acidified to pH 5 by addition of HCl (1 M, 3 mL) and extracted with Et_2O (3 x 15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give **67**

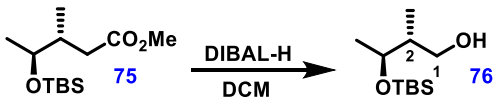
as a colourless oil (75 mg, 72%); δ_H (400 MHz, $CDCl_3$) 5.71 (1 H, m, 6-H), 4.98 (2 H, m, 7-H_2), 3.96 (1 H, m, 3-H), 2.45 (2 H, m, 2-H_2), 2.18 (1 H, m, 5-*H*H), 1.88 (1 H, m, 5-*H*H), 1.59 (1 H, m, 4-H) 0.86 (3 H, d, *J* 7.0, 4-Me); δ_C (100 MHz, $CDCl_3$) 176.8 (C-1), 136.7 (C-6), 116.5 (C-7), 70.5 (C-3), 38.4 (C-2), 38.0 (C-4), 37.4 (C-5), 13.8 (CH_3). Data in accordance with the literature.

Methyl (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylbutanoate 75



Alcohol **74** (600 mg, 4.54 mmol), imidazole (463 mg, 6.91 mmol) and DMAP (55 mg, 0.45 mmol) were dissolved in anhydrous DCM (15 mL) under N_2. TBSCl (1.23 g, 8.17 mmol) was added and the reaction stirred for 16 h. The reaction was quenched with H_2O (*ca.* 10 mL) and extracted with DCM (3 x 15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (2% EtOAc in petrol) to give ester **75** as a colourless oil (1.08 g, 96%); $[\alpha]_D^{24}$ +35.4 (*c* 1.3, CHCl3), lit.[55] $[\alpha]_D^{24}$ (c 1.25, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.98 (1 H, dq, *J* 7.0, 6.0, 3-H), 3.63 (3 H, s, OMe), 2.47 (1 H, pent, *J* 7.0, 2-H), 1.10 (3 H, d, *J* 6.0, 4-H_3), 1.06 (3 H, d, *J* 7.0, 2-CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.03 (3 H, s, $SiCH_3$), 0.00 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$): 70.2 (C-3), 51.4 (OCH_3), 48.1 (C-2), 25.7 ($SiC(\underline{C}H_3)_3$), 20.6 (C-4), 17.9 ($Si\underline{C}(CH_3)_3$), 12.7 (2-CH_3), -4.3 ($Si(CH_3)_2$), -5.1 ($Si(CH_3)_2$). Data in accordance with the literature.[206]

(2*R*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylbutan-1-ol 76



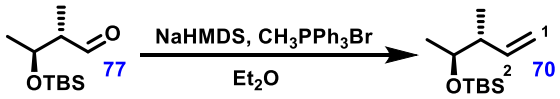
Ester **75** (500 mg, 2.15 mmol) was dissolved in DCM (6 mL) and cooled to -78 °C and DIBAL-H (1 M in DCM, 4.52 mL, 4.52 mmol) was added dropwise under N_2. The reaction mixture was stirred for 2 h at RT. Rochelle's salt (10 mL) was added and the reaction mixture was stirred vigorously overnight before being extracted with DCM (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give **76** as a colourless oil (447 mg, 95%); $[\alpha]_D^{24}$ = +12.0 (*c* 1.0, $CHCl_3$), lit.[56] $[\alpha]_D^{24}$ = +18.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.72 (1 H, m, 1-*H*H), 3.66 (1 H, m, 3-H), 2.73 (1 H, m, 1-H*H*), 1.52 (1 H, m, OH), 1.12 (3 H, d, *J* 7.0, 4-H_3), 0.87 (3 H, d, *J* 7.0, 2-CH_3), 0.80 (9 H, s, $SiC(CH_3)_3$), 0.00 (3 H, s,

$SiCH_3$), 0.00 (3 H, s, $SiCH_3$); δ_C (400 MHz, $CDCl_3$): 74.1 (C-3), 65.9 (C-1), 41.7 (C-2), 25.8 ($SiC(\underline{C}H_3)_3$), 22.2 (C-4), 17.9 ($SiC(\underline{C}H_3)_3$), 14.7 (2-CH_3), -4.2 ($Si(CH_3)_2$), -5.0 ($Si(CH_3)_2$). Data in accordance with the literature.[207]

(2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-methylbutanal 77

Alcohol **76** (300 mg, 1.47 mmol) was dissolved in anhydrous DCM (5 mL) and TEMPO (459 mg, 2.94 mmol) was added followed by BAIB (711 mg, 2.21 mmol) under N_2. The reaction mixture was stirred at RT for 3 h before the reaction was quenched with sat. aq. $Na_2S_2O_3$ (5 mL) and extracted with DCM (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (0-5% EtOAc in petrol) to give aldehyde **77** as a pale yellow oil (250 mg, 83%); $[\alpha]_D^{24}$ = +43 (*c* 1.0, $CHCl_3$), lit.[56] $[\alpha]_D^{24}$ = +47 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 9.69 (1 H, d, *J* 2.5, 1-H), 3.96 (1 H, m, 3-H), 2.30 (1 H, app. pd, *J* 6.0, 2.5, 2-H), 1.15 (3 H, d, *J* 6.0, 4-H_3), 1.00 (3 H, d, *J* 6.0, 2-CH_3), 0.80 (9 H, s, $SiC(CH_3)_3$), 0.00 (3 H, s, $SiCH_3$), -0.01 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 205.2 (C-1), 69.9 (C-3), 53.7 (C-2), 25.7 ($SiC(\underline{C}H_3)_3$), 21.8 (C-4), 18.0 ($Si\underline{C}(CH_3)_3$), 10.6 (2-CH_3), -4.2 ($SiCH_3$), -5.0 ($SiCH_3$). Data in accordance with the literature.[208]

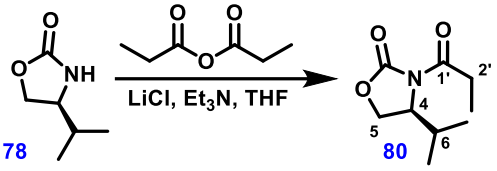
(3*R*,4*S*)-4-(*tert* Butyldimethylsilyloxy)-3-methylpent-1-ene 70



CH_3PPh_3Br (1.16 g, 3.25 mmol) was suspended anhydrous Et_2O (10 mL) and cooled to -78 °C under N_2. NaHMDS (1 M in Et_2O, 3.33 mL, 3.33 mmol) was added and stirred for 1 h at this temperature. Aldehyde **77** (600 mg, 2.78 mmol) was added dropwise and the reaction mixture stirred for 16 h at RT. The reaction mixture was quenched with sat. aq. NH_4Cl (10 mL), extracted with Et_2O (3 x 10 mL) and the combined organic extracts were dried ($MgSO_4$). The solvent was removed *in vacuo* to give a pale yellow oil which was purified by column chromatography (1% EtOAc in petrol) to give **70** as a colourless oil (446 mg, 64%); $[\alpha]_D^{23.6}$ = +7.5 (*c* 2.0, $CHCl_3$), lit.[54] $[\alpha]_D^{26.6}$ = +8.0 (*c* 2.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.75 (1 H, ddd, *J* 17.0, 10.5, 7.5, 2-H), 4.96 (1 H, m, 1-*H*H), 4.92 (1 H, m, 1-*H*H), 3.67 (1 H, qd, *J* 6.0, 4.5, 4-H),

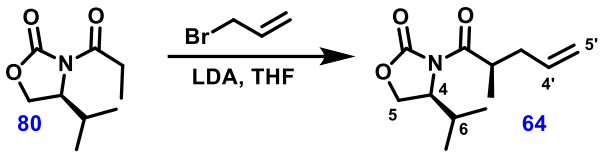
2.13 (1 H, m, 3-H), 1.01 (3 H, d, *J* 6.5, 5-H_3), 0.95 (3 H, d, *J* 6.5, 3-CH_3), 0.85 (9 H, s, $SiC(CH_3)_3$), 0.00 (3 H, s, $SiCH_3$), 0.00 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 141.2 (C-2), 114.2 (C-1), 71.7 (C-4), 45.4 (C-3), 25.9 ($SiC(\underline{C}H_3)_3$), 20.6 (C-5), 18.1 ($Si\underline{C}(CH_3)_3$), 15.5 (3-CH_3), -4.3 ($SiCH_3$), -4.8 ($SiCH_3$). Data in accordance with the literature.[209]

(*S*)-4-Isopropyl-3-propionyloxazolidin-2-one 80



Auxiliary **78** (1.00 g, 7.74 mmol) was dissolved in anhydrous THF (25 mL) and cooled to 0 °C under N_2. LiCl (0.36 g, 8.51 mmol) was added followed by Et_3N (1.41 mL, 10.1 mmol) and the resulting mixture stirred for 30 min. Propionic anhydride (1.04 mL, 8.13 mmol) was added dropwise and the reaction was warmed slowly to RT before stirring for 1.5 h. After this time the reaction was quenched by addition of NaCl (1 M, 10 mL), extracted with EtOAc (3 x 15 mL) and the combined extracts washed HCl (1 M, 10 mL) before being dried ($MgSO_4$). The solvent was removed *in vacuo* to give a colourless oil which was purified by column chromatography (40% EtOAc in petrol) to give **80** as a colourless oil (1.33 g, 93%). δ_H (400 MHz, $CDCl_3$) 4.37 (1 H, dt, *J* 8.0, 3.5, 4-H), 4.20 (1 H, m, 5-*H*H), 4.14 (1 H, dd, *J* 9.0, 3.0. 5-H*H*), 2.92 (1 H, dq, *J* 18.0, 7.5, 2'-*H*H), 2.83 (1 H, dq, *J* 18.0, 7.5, 2'-H*H*), 2.31 (1 H, ddq, *J* 11.0, 7.0, 4.0, 3.5, 6-H), 1.10 (3 H, t, *J* 7.0, 3'-H_3), 0.86 (3 H, d, *J* 7.0, 6-CH_3), 0.82 (3 H, d, *J* 7.0, 6-CH_3); δ_C (100 MHz, $CDCl_3$) 174.1 (C-1'), 154.1 (C-2), 63.4 (C-5), 58.4 (C-4), 29.2 (C-2'), 28.4 (C-6), 18.0 (6-CH_3), 14.7 (6-CH_3), 8.5 (C-3'). Data in accordance with the literature.[210]

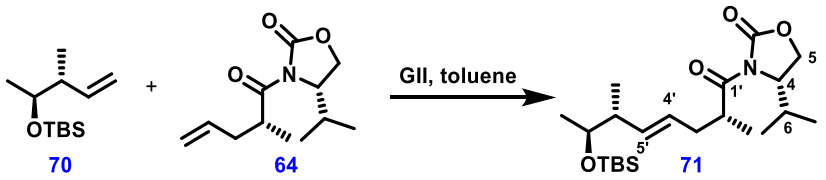
(*S*)-4-Isopropyl-3-((*R*)-2-methylpent-4-enoyl)oxazolidin-2-one 64



DIPA (1.59 mL, 11.34 mmol) was dissolved in anhydrous THF (10 mL) and cooled to -78 °C. nBuLi (1.52 M in hexanes, 7.46 mL, 11.34 mmol) was added dropwise under N_2 and the reaction stirred at this temperature for 30 mins. **80** (1.00 g, 5.40 mmol) in anhydrous THF (10 mL) was added dropwise at -78 °C. After 30 mins allyl bromide (1.96 g, 16.20 mmol) was

added and the reaction stirred at 0 °C for 4 h after which time sat. aq. NH_4Cl (15 mL) was added and the reaction mixture extracted with DCM (3 x 15 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a yellow oil, which was purified by column chromatography (40% EtOAc in petrol) to give **64** as a colourless oil (0.89 g, 73%); $[\alpha]_D^{26}$ = +59 (*c* 1.0, $CHCl_3$), lit.[57] $[\alpha]_D^{20}$ = +62.3 (*c* 1.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.72 (1 H, ddt, *J* 17.0, 10.0, 7.0, 4'-H), 5.02 (1 H, m, 5'-H), 4.98 (1 H, m, 5'-H), 4.39 (1 H, dt, *J* 9.0, 3.0, 4-H), 4.19 (1 H, m, 5-*H*H), 4.12 (1 H, dd, *J* 9.0, 3.0, 5-H*H*), 3.82 (1 H, app. sextet, *J* 7.0, 2'-H), 2.44 (1 H, ddt, *J* 14.0, 7.0, 1.0, 3'-*H*H), 2.25 (1 H, ddq, *J* 10.0, 7.0, 3.0, 6-H), 2.13 (1 H, ddt, *J* 14.0, 7.0, 1.0, 3'-H*H*), 1.08 (3 H, d, *J* 7.0, 2'-CH_3), 0.84 (3 H, d, *J* 7.0, 6-CH_3), 0.80 (3 H, d, *J* 7.0, 6-CH_3); δ_C (100 MHz, $CDCl_3$) 176.5 (C-1'), 153.7 (C-2), 135.3 (C-4'), 117.1 (C-5'), 63.1 (C-5), 58.5 (C-4), 38.2 (C-3'), 37.2 (C-2'), 28.5 (C-6), 18.0 (6-CH_3), 16.2 (2'-CH_3), 14.7 (6-CH_3). Data in accordance with the literature.[211]

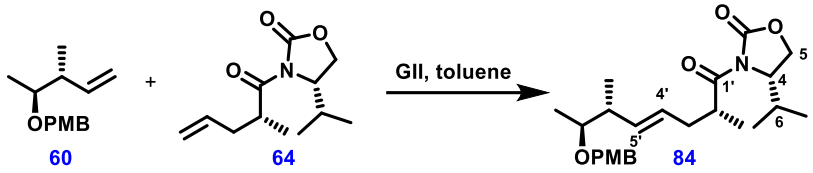
(*S*)-3-((2*R*,6*R*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-2,6-dimethyloct-4-enoyl)-4-isopropyloxazolidin-2-one 71



Terminal alkene **64** (52 mg, 0.23 mmol) and **70** (200 mg, 0.93 mmol) were added simultaneously *via* syringe to a stirring solution of G2 (19 mg, 0.02 mmol) in anhydrous toluene (2.5 mL) under N_2 at RT. The reaction was stirred for 16 h at 80 °C after which time it was cooled to RT, filtered through silica and concentrated *in vacuo* to give a brown liquid which was purified by column chromatography (5-10% EtOAc in petrol) to give **71** as a colourless oil (52 mg, 53%); ν_{max} (neat)/cm^{-1} 3010, 2956, 2874, 1742 (C=O), 1635, 1451, 1427, 1368, 1229; $[\alpha]_D^{24}$=+24 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.42 (1 H, dd, *J* 15.5, 7.5, 5'-H), 5.33 (1 H, m, 4'-H), 4.43 (1 H, ddd, *J* 8.5, 4.0, 3.0, 4-H), 4.23 (1 H, app. t, *J* 8.5, 5-H*H*), 4.12 (1 H, dd, *J* 9.0, 3.0, 5-*H*H), 3.77 (1 H, m, 2'-H), 3.65 (1 H, qd, *J* 6.0, 4.0, 7'-H), 2.44 (1 H, m, 3'-*H*H), 2.31 (1 H, m, 6-H), 2.30 (1 H, m, 6'-H) 2.08 (1 H, dt, *J* 14.0, 7.0, 3'-*H*H), 1.10 (3 H, d, *J* 7.0, 2'-CH_3), 0.99 (3 H, d, *J* 7.0, 8'-H_3), 0.91 (3 H, d, *J* 7.0, 6'-CH_3), 0.88 (3 H, d, *J* 7.0, 6-CH_3), 0.86 (9 H, s, $C(CH_3)_3$), 0.83 (3 H, d, *J* 7.0, 6-CH_3), 0.00 (3 H, s, $SiCH_3$), 0.00 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 176.6 (C-1'), 153.7 (C-2), 135.7 (C-5'), 126.7 (C-4'), 71.8 (C-7'), 63.1 (C-5), 58.4 (C-4),

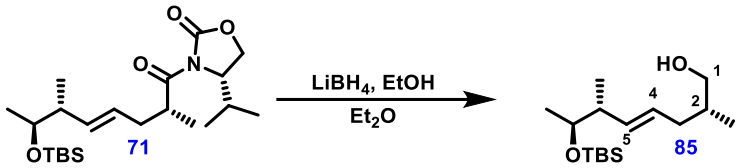
44.2 (C-6'), 37.8 (C-2'), 37.1 (C-3'), 28.5 (C-6), 25.9 (SiC($\underline{C}H_3)_3$), 20.4 (C-8'), 18.1 (Si$\underline{C}(CH_3)_3$), 18.0 (6-CH_3), 16.0 (2'-CH_3), 15.8 (6'-CH_3), 14.7 (6-CH_3), -4.4 ($SiCH_3$), -4.8 ($SiCH_3$); Found (ESI) 434.2693 $[M+Na]^+$, ($C_{22}H_{41}NaNO_4Si$ requires 434.2805).

(*S*)-4-Isopropyl-3-((2*R*,6*R*,7*S*,*E*)-7-((4-methoxybenzyl)oxy)-2,6-dimethyloct-4-enoyl)oxazolidin-2-one 84



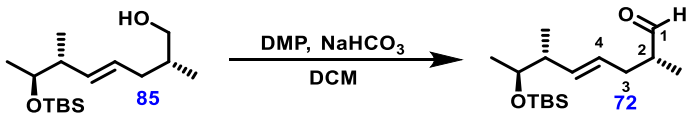
Terminal alkene **64** (52 mg, 0.23 mmol) and **60** (200 mg, 0.91 mmol) were added simultaneously *via* syringe to a stirring solution of G2 (19 mg, 0.02 mmol) in anhydrous toluene (2.5 mL) under N_2 at RT. The reaction was stirred for 16 h at 80 °C after which time it was cooled to RT, filtered through silica and concentrated *in vacuo* to give a brown liquid. This was purified by column chromatography (5-20% EtOAc in petrol) to give **84** as a colourless oil (38 mg, 40%); ν_{max} (neat)/cm^{-1} 3021, 2946, 2894, 1740 (C=O), 1632, 1459, 1424, 1369, 1229; $[\alpha]_D^{24}$ = +25 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.19 (2 H, d, *J* 8.5, Ar-H), 6.80 (2 H, d, *J* 8.5, Ar-H), 5.40 (1 H, dd, *J* 15.5, 7.0, 5'-H), 5.33 (1 H, m, 4'-H), 4.41 (1 H, d, *J* 11.0, OC*H*H), 4.37 (1 H, m, 4-H), 4.32 (1 H, d, *J* 11.0, OCH*H*), 4.19 (1 H, m, 5-H*H*), 4.12 (1 H, dd, *J* 9.0, 3.0, 5-*H*H), 3.73 (3 H, s, OMe), 3.73 (1 H, m, 2'-H), 3.31 (1 H, m, 7'-H), 2.41 (1 H, m, 3'-*H*H), 2.27 (1 H, m, 6-H), 2.23 (1 H, m, 6'-H) 2.06 (1 H, app. dt, *J* 14.0, 7.0, 3'-*H*H), 1.05 (3 H, d, *J* 7.0, 2'-CH_3), 1.00 (3 H, d, *J* 7.0, 8'-H_3), 0.90 (3 H, d, *J* 7.0, 6'-CH_3), 0.83 (3 H, d, *J* 7.0, 6-CH_3), 0.79 (3 H, d, *J* 7.0, 6-CH_3); δ_C (125 MHz, $CDCl_3$) 176.6 (C-1'), 159.0 (Ar-C), 153.7 (C-2), 135.7 (C-5'), 131.2 (Ar-C), 129.1 (Ar-C), 126.7 (C-4'), 113.7 (Ar-C), 78.0 (C-7'), 70.3 (OCH_2), 63.1 (C-5), 58.4 (C-4), 55.3 (OCH_3), 41.5 (C-6'), 37.8 (C-2'), 37.1 (C-3'), 28.5 (C-6), 18.0 (6-CH_3), 16.1 (C-8'), 16.0 (2'-CH_3), 15.2 (6'-CH_3), 14.7 (6-CH_3); Found (ESI) 440.2313 $[M+Na]^+$, ($C_{22}H_{41}NaNO_4Si$ requires 440.2407).

(2*R*,6*R*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-2,6-dimethyloct-4-en-1-ol 85



Alkene **71** (150 mg, 0.36 mmol) was dissolved in anhydrous Et_2O (5 mL) and cooled to 0°C under N_2. To this solution was added EtOH (0.32 mL, 0.55 mmol) followed by dropwise addition of $LiBH_4$ (0.28 mL, 0.55 mmol). The solution was stirred at 0°C for 1 h, followed by 3 h at RT. Aq. NaOH (1 M, 3 mL) was added and the reaction mixture extracted with Et_2O (3 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale yellow oil. This was purified by column chromatography (40% EtOAc in petrol) to give **85** as a pale yellow oil (92 mg, 88%); ν_{max} (neat)/cm^{-1} 3339 (OH), 2957, 2928, 2857, 1462, 1374, 1252; $[\alpha]_D^{25}$=+ 80 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.36 (2 H, m, 4-H & 5-H), 3.65 (1 H, m, 7-H), 3.47 (1 H, m, 1-*H*H), 3.42 (1 H, m, 1-H*H*), 2.08 (1 H, m, 6-H), 2.04 (1 H, m, 3-*H*H), 1.89 (1 H, m, 3-*H*H), 1.66 (1 H, m, 2-H), 1.00 (3 H, d, *J* 6.5, 8-H_3), 0.93 (3 H, d, *J* 6.5, 6-H_3), 0.88 (3 H, d, *J* 6.5, 2-CH_3), 0.85 (9 H, s, $SiC(CH_3)_3$), 0.03 (3 H, s, $SiCH_3$), 0.03 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 134.6 (C-5), 128.2 (C-4), 72.1 (C-7), 68.2 (C-1), 44.4 (C-6), 36.8 (C-3), 36.1 (C-2), 26.1 ($SiC(CH_3)_3$), 26.0 ($SiC(CH_3)_3$), 20.8 (C-8), 16.5 (2-CH_3), 16.2 (6-CH_3), -4.2 ($SiCH_3$), -4.7 ($SiCH_3$); Found (ESI): 309.2221 $[M+Na]^+$, ($C_{16}H_{34}NaO_2Si$ requires 309.2220).

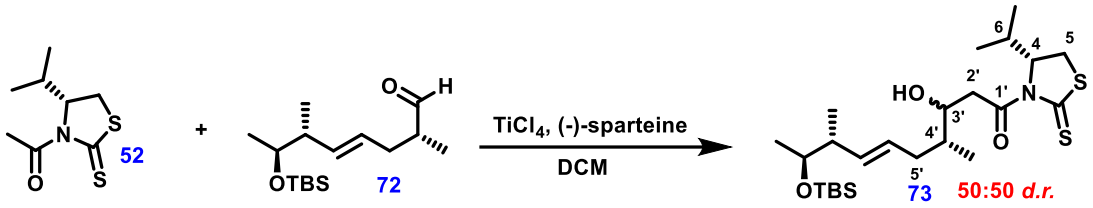
(2*R*,6*R*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-2,6-dimethyloct-4-enal 72



Alcohol **85** (140 mg, 0.52 mmol) was dissolved in anhydrous DCM (15 mL) and $NaHCO_3$ was added and the reaction cooled to 0 °C. DMP (0.3 M in DCM, 1.8 mL, 0.68 mmol) was added dropwise and the reaction mixture stirred at RT for 1.5 h, then quenched by addition of sat. aq. $Na_2S_2O_3$ (10 mL). The reaction mixture was then extracted with DCM (3 x 10 mL), the combined organic extracts dried ($MgSO_4$) and the solvent removed *in vacuo* to give a white oily solid. This was purified by column chromatography (5% EtOAc in petrol) to give aldehyde **72** as a colourless oil (125 mg, 89%); ν_{max} (neat)/cm^{-1} 2957, 2928, 2956, 1727, 1461, 1374, 1252, 1093, 1029; $[\alpha]_D^{25}$=+ 120 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 9.65 (1 H, d, *J* 1.5, 1-H),

5.46 (1 H, ddt, *J* 15.5, 8.0, 1.0, 4-H), 5.33 (1 H, m, 5-H), 3.67 (1 H, qd, *J* 6.0, 4.0, 7-H), 2.40 (2 H, m, 2-H & 3*H*H), 1.95 (2 H, m, 6-H, 3*H*H), 1.09 (3 H, d, *J* 7.0, 2-CH_3), 1.02 (3 H, d, *J* 7.0, 8-H_3), 0.95 (3 H, d, *J* 7.0, 6-H_3), 0.88 (9 H, s, $C(CH_3)_3$), 0.03 (3 H, s, $SiCH_3$), 0.03 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 205.0 (C-1), 135.7 (C-4), 126.0 (C-5), 71.8 (C-7), 46.3 (C-2), 44.3 (C-6), 33.9 (C-3), 25.9 ($SiC(\underline{C}H_3)_3$), 20.8 (C-8), 18.1 ($Si\underline{C}(CH_3)_3$), 16.2 (6-CH_3), 13.1 (2-CH_3), -4.3 ($SiCH_3$), -4.8 ($SiCH_3$); Found (ESI): 307.2065 $[M+Na]^+$, ($C_{16}H_{32}NaO_2Si$ requires 307.2064).

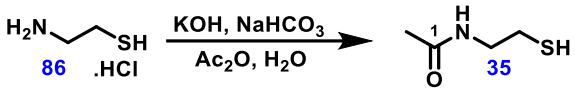
(3*S*,4*R*,8*R*,9*S*,*E*)-9-((*tert*-Butyldimethylsilyl)oxy)-3-hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)-4,8-dimethyldec-6-en-1-one 73



Auxiliary **52** (268 mg, 1.32 mmol) was dissolved in anhydrous DCM (10 mL) under N_2 and the system degassed (3 x 5 mins). The reaction mixture was cooled to 0 °C and $TiCl_4$ (2.64 mL, 2.64 mmol, 1 M in toluene) was added dropwise. After stirring for 10 mins, the reaction mixture was cooled to -40 °C and (-)-sparteine (0.17 mL, 1.32 mmol) was added dropwise with vigourous stirring. After stirring for 1 h at -40 °C, the reaction was cooled to -78 °C and aldehyde **72** (125 mg, 0.44 mmol) in anhydrous DCM (1 mL) was added. The reaction was stirred for a further 45 mins at this temperature before the addition of phosphonate buffer solution (*ca.* 10 mL). The reaction mixture was extracted with DCM (3 x 10 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a bright yellow oil. This was purified by column chromatography (10-20% EtOAc in petrol) to give **73** as a yellow oil (137 mg, 64%); ν_{max} (neat)/cm^{-1} 2959, 2928, 2856, 1687, 1462, 1371, 1306, 1253, 1159, 1092, 1033; δ_H (400 MHz, $CDCl_3$) 5.38 (2 H, m, 6'-H and 7'-H), 5.16 (1 H, ddd, *J* 8.0, 6.0, 1.0, 4-H), 4.10-4.00 (1 H, m, 3'-H), 3.69 (1 H, qd, *J* 6.0, 4.0, 9'-H), 3.60 (1 H, m, 2'-H*H*), 3.52 (1 H, dd, *J* 11.5, 8.0, 5-H*H*), 3.26-3.12 (1 H, m, 2'-*H*H), 3.03 (1 H, dd, *J* 11.5, 1.0, 5-*H*H), 2.37 (1 H, m, 6-H), 2.21 (1 H, m, 5'-*H*H), 2.14 (1 H, dq, *J* 11.0, 4.0, 3.5, 8'-H), 1.94 (1 H, m, 4'-H), 1.74-1.60 (1 H, m, 5'-H*H*), 1.07 (3 H, d, *J* 7.0, 6-CH_3), 1.02 (3 H, d, *J* 6.5, 10'-CH_3), 0.98 (3 H, d, *J* 7.0, 6-CH_3), 0.96 (3 H, d, *J* 7.0, 8'-CH_3), 0.91 (3 H, d, *J* 7.0, 4'-CH_3), 0.88 (9 H, s, $SiC(\underline{C}H_3)_3$), 0.03 (6 H, s, $SiCH_3$ x 2); δ_H (100 MHz, $CDCl_3$) 203.2 (C-1'), 173.9 (C-2), 134.8 (C-6'), 128.1 (C-7'), 72.1 (C-9'), 71.6 (C-4), 71.5 (C-3'), 44.4 (C-8'), 42.5 (C-2'), 38.7 (C-5'), 36.7 (C-4'), 31.0 (C-6), 30.8

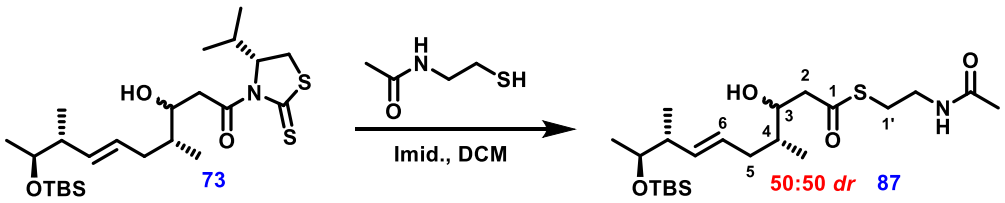
(C-5), 26.1 (SiC(CH$_3$)$_3$), 20.7 (10'-CH$_3$), 19.2 (6-CH$_3$), 18.3 (SiC(CH$_3$)$_3$), 18.0 (6-CH$_3$), 16.1 (8'-CH$_3$), 15.4 (4'-CH$_3$), -4.2 (SiCH$_3$), -4.6 (SiCH$_3$); Found (ESI) 488.2684 [M+Na]$^+$, ($C_{24}H_{45}NaNO_3S_2Si$ requires 488.2683).

_N_-acetylcysteamine 35



Cysteamine hydrochloride **86** (1.00 g, 8.80 mmol), KOH (0.49 g, 8.80 mmol) and NaHCO$_3$ (2.22 g, 26.4 mmol) were dissolved in water (50 mL). Ac$_2$O (0.82 mL, 8.80 mmol) was added dropwise and the reaction stirred at RT for 1 h. The pH was adjusted to *ca*. pH 7 and the reaction mixture extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried (MgSO$_4$) and the solvent removed *in vacuo* to give **35** as a colourless oil (1.02 g, 97%); δ_H (400 MHz, CDCl$_3$) 6.09 (1 H, br s, NH), 3.41 (2 H, app. q, *J* 6.5, 3-H$_2$), 2.65 (2 H, dt, *J* 8.5, 6.5, 4-H$_2$), 1.99 (3 H, s, 1-CH$_3$), 1.35 (1 H, t, *J* 8.5, SH); δ_C (100 MHz, CDCl$_3$) 170.5 (C-1), 42.6 (C-3), 24.6 (C-2), 23.3 (1-CH$_3$). Data in accordance with the literature.[90]

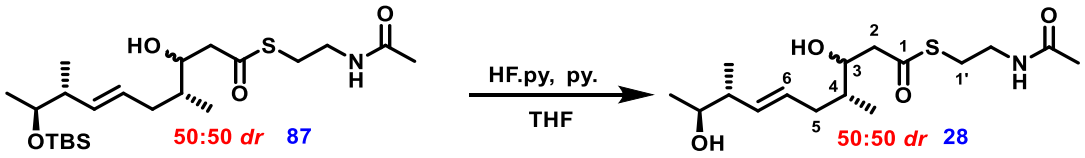
_S_-(2-acetamidoethyl)(4_R_,8_R_,9_S_,_E_)-9-((_tert_-butyldimethylsilyl)oxy)-3-hydroxy-4,8-dimethyldec-6-enethioate 87



Alkene **73** (100 mg, 0.20 mmol) was dissolved in anhydrous DCM (1 mL) and imidazole (40 mg, 0.60 mmol) and HSNAC (27 mg, 0.23 mmol) were added under N$_2$. The reaction mixture was stirred at RT for 2.5 h. The solvent was removed *in vacuo* and the residue applied directly to a silica column containing a layer of CuSO$_4$ impregnated silica. The crude material was purified (20-100% EtOAc in petrol) to give **87** as a colourless oil (55 mg, 62%); ν_{max} (neat)/cm^{-1} 3299 (OH), 2957, 2958, 2929, 2857, 1659 (C=O), 1551, 1462, 1374, 1253, 1097,1031; δ_H (400 MHz, CDCl$_3$) 5.85 (1 H, brs, NH), 5.38 (1 H, m, 6-H), 5.34 (1 H, m, 7-H), 4.00-3.90 (1 H, m, 3-H), 3.65 (1 H, qd, *J* 6.0, 4.0, 9-H), 3.42 (2 H, app. q, *J* 6.5, 2'-H$_2$), 3.03 (2 H, app. hept, *J* 7.5, 6.5, 4.0, 2-H$_2$), 2.68 (2 H, m, 1'-H$_2$), 2.16 (1 H, m, 5-*H*H), 2.11 (1 H, m, 8-H), 1.93 (3 H, s, 4'-H$_3$), 1.90 (1 H, m, 4-H), 1.65-1.58 (1 H, m, 5-*H*H), 1.00 (3 H, d, *J* 6.5, 10-H$_3$), 0.95 (3 H, d, *J* 6.5, 8-

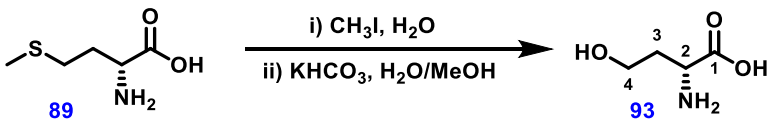
CH_3), 0.89 (3 H, d, *J* 6.5, 4-CH_3), 0.85 (9 H, s, $SiC(CH_3)_3$), 0.00 (3 H, s, $SiCH_3$), 0.00 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 199.9 (C-1), 170.5 (C-3'), 135.0 (C-6), 130.0 (C-7), 72.6 (C-3), 71.6 (C-9), 48.8 (C-1'), 44.4 (C-8), 39.5 (C-2'), 36.6 (C-4), 36.1 (C-5), 29.0 (C-2), 26.0 ($SiC(\underline{C}H_3)_3$), 23.4 (C-4'), 20.9 (C-10), 18.3 ($Si\underline{C}(CH_3)_3$), 16.4 (8-CH_3), 14.1 (4-CH_3), -4.2 ($SiCH_3$), -4.7 ($SiCH_3$); Found (ESI) 446.2758 $[M+H]^+$, ($C_{22}H_{43}NO_4SSi$ requires 446.2755).

_S_-(2-acetamidoethyl) (4_R_,8_R_,9_S_,_E_)-3,9-dihydroxy-4,8-dimethyldec-6-enethioate 28



To a solution of **87** (50 mg, 0.11 mmol) in pyridine (0.20 mL) and anhydrous THF (2 mL) was added HF.pyridine (0.62 mL, 0.24 mmol) under N_2. The reaction mixture was stirred at RT for 5 h. The reaction was then diluted with Et_2O (2 mL), washed with H_2O (*ca.* 3 mL), sat. aq. NaCl (*ca.* 3 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give a colourless oil that was purified by column chromatography (3-6% MeOH in DCM), to give **28** as a colourless oil (29 mg, 77%); ν_{max} (neat)/cm^{-1} 3300 (OH), 2966, 2928, 1658 (C=O), 1552, 1439, 1405, 1375, 1290, 1093, 1047, 1005; δ_H (400 MHz, $CDCl_3$) 6.02 (1 H, brs, NH), 5.46 (1 H, dt, *J* 15.0, 7.0, 6-H), 5.31 (1 H, ddd, *J* 15.0, 8.5, 3.0, 7-H), 3.98 (1 H, m, 3-H), 3.46 (1 H, app. p, *J* 6.0, 9-H), 3.38 (2 H, app. p, *J* 6.5, 2'-H_2), 2.98 (2 H, m, 2-H_2), 2.65 (2 H, m, 1'-H_2), 2.13 (1 H, m, 5-*H*H), 2.00 (1 H, m, 8-H), 1.91 (1 H, m, 4-H), 1.90 (3 H, s, 4'-H_3), 1.65 (1 H, m, 5-*H*H), 1.10 (3 H, d, *J* 6.5, 10-H_3), 0.92 (3 H, d, *J* 6.5, 8-CH_3), 0.86 (3 H, d, *J* 6.5, 4-CH_3); δ_C (100 MHz, $CDCl_3$) 199.9 (C-1), 170.7 (C-3'), 134.4 (C-6), 130.2 (C-7), 71.5 (C-9), 71.3 (C-3), 48.9 (C-1'), 48.2 (C-8), 45.2 (C-4), 39.4 (C-2'), 35.9 (C-5), 29.1 (C-2), 23.3 (C-4'), 20.5 (C-10), 16.9 (8-CH_3), 15.6 (4-CH_3); Found (ESI) 354.1720 $[M+Na]^+$, ($C_{16}H_{29}NNaO_4S$ requires 354.1709).

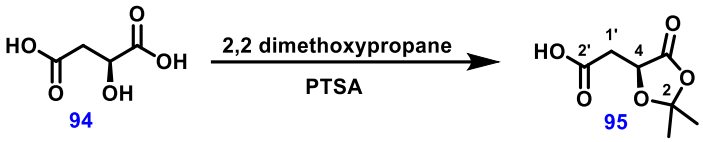
D-homoserine 93



D-methionine **89** (1.00 g, 6.70 mmol) was dissolved in H_2O (15 mL) and CH_3I (1.25 mL, 20.1 mmol) was added dropwise. The reaction mixture was stirred for 24 h at RT. The solvent was removed *in vacuo* until *ca.* 10 mL remained and $KHCO_3$ (0.93 g, 6.70 mmol) in H_2O (3.50 mL)

was added dropwise over 1 h to maintain pH 6. The solution was heated to reflux for 10 h, after which time the solvent was removed *in vacuo*. A solution of MeOH:H_2O (100:1, 15 mL) was added, followed by the dropwise addition of conc. HCl (*ca.* 2 mL) to adjust the pH to 6. The reaction mixture was filtered while it was still hot, and the solvent concentrated to *ca.* 5 mL. The resulting colourless solution was crystallised by freezing to give **93** as a white solid (630 mg, 79%); lit. $[\alpha]_D^{25}$=+8.7; $[\alpha]_D^{24}$=+8.8 (*c* 5, H_2O); δ_H (400 MHz, D_2O) 4.07 (2 H, m, 4-H_2), 2.69 (1 H, t, *J* 7.5, 2-H), 2.19 (1 H, m, 3-*H*H), 2.10 (1 H, m, 3-*H*H). Data in accordance with the literature.[199]

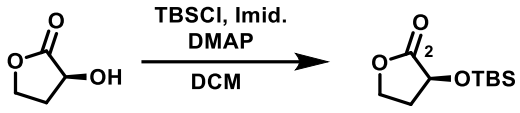
(*S*)-2-(2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid 95



94 95

L-Malic acid **94** (5.00 g, 37.3 mmol) was dissolved in 2,2 dimethoxypropane (19 mL, 149.2 mmol) at RT. PTSA (71 mg, 0.37 mmol) was added and the reaction mixture was stirred for 3 h. The reaction was quenched by addition of H_2O (25 mL) containing $NaHCO_3$ (31 mg) followed by extraction with DCM (5 x 25 mL), dried (Na_2SO_4) and the solvent was removed *in vacuo*. The resulting colourless oil was redissolved in Et_2O (10 mL) and hexane (150 mL) and half the solvent removed *in vacuo*. The resulting colourless crystals were filtered to give **95** (6.20 g, 96%); δ_H (400 MHz, $CDCl_3$) 4.75 (1 H, dd, *J* 6.5, 4.0, 4-H), 3.03 (1 H, dd, *J* 17.0, 4.0, 1'-*H*H), 2.88 (1 H, dd, *J* 17.0, 4.0, 1'-*H*H), 1.66 (3 H, s, 2-CH_3), 1.60 (3 H, s, 2-CH_3); δ_C (100 MHz, $CDCl_3$); 174.5 (C-2'), 171.8 (C-5), 111.4 (C-2), 70.4 (C-4), 36.0 (C-1'),26.8 (2-CH_3), 25.8 (2-CH_3). Data in accordance with the literature.[200]

(*S*)-3-(*tert*-Butyldimethylsilyloxy)dihydrofuran-2(3H)-one 97

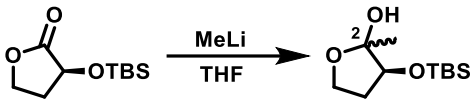


90 97

Lactone **90** (200 mg, 1.96 mmol) was dissolved in anhydrous DCM (10 mL) under N_2. Imidazole (200 mg, 2.94 mmol), TBSCl (532 mg, 3.53 mmol) and DMAP (24.0 mg, 0.20 mmol) were added and the reaction stirred for 16 h at RT. The reaction was quenched by addition of H_2O (10 mL) and extracted with DCM (3 x 10 mL). The combined organic extracts were

dried ($MgSO_4$) and the solvent removed *in vacuo* to give **97** as a colourless oil (413 mg, 97%); ν_{max} (neat)/cm^{-1} 2935, 2864, 1784, 1151, 1021, 998; $[\alpha]_D^{25}$= -10 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.38 (2 H, m, 5-*H*H + 3-H), 4.18 (1 H, tdd, *J* 9.0, 6.5, 0.5, 5-*H*H), 2.45 (1 H, dddd, *J* 12.5, 7.5, 6.5, 3.5, 4-*H*H), 2.21 (1 H, dddd, *J* 12.5, 9.0, 8.5, 8.0, 4-*H*H), 0.90 (9 H, s, $SiC(CH_3)_3$), 0.16 (3 H, s, $SiCH_3$), 0.14 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 176.0 (C-2), 68.4 (C-3), 64.9 (C-5), 32.5 (C-4), 25.8 ($SiC(\underline{C}H_3)_3$), 18.4 ($Si\underline{C}(CH_3)_3$), -4.6 ($SiCH_3$), -5.1 ($SiCH_3$); Found (ESI): 239.1077 $[M+Na]^+$, ($C_{10}H_{20}NaO_3SiNa$ requires 239.1074).

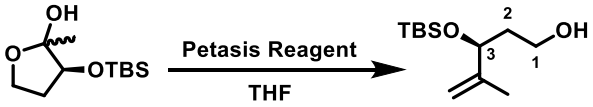
(3*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-methyltetrahydrofuran-2-ol 98



97 98

Lactone **97** (2.40 g, 11.2 mmol) was dissolved in anhydrous THF (25 mL) at -78 °C under N_2. MeLi (1.6 M in Et_2O, 7.69 mL, 12.3 mmol) was added and the reaction mixture was stirred for 3 h at -78 °C followed by addition of aqueous NH_4Cl (15 mL). The reaction mixture was extracted with EtOAc (3 x 20 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale yellow oil. This was purified by column chromatography (20% EtOAc in petrol) to give lactol **98** as a colourless oil (2.1 g, 81%); ν_{max} (neat)/cm^{-1} 3434, 2961, 2929, 2864, 1252, 1103, 1088, 1054, 1021, 1005; δ_H (400 MHz, $CDCl_3$) 4.03 (1 H, m, 3-H), 4.00 (1 H, m, 5-*H*H), 3.80 (1 H, m, 5-*H*H), 2.14 (1 H, m, 4-*H*H), 1.81 (1 H, m, 4-*H*H), 1.39 (3 H, s, CH_3), 0.91 (9 H, s, $SiC(CH_3)_3$), 0.12 (6 H, s, $SiCH_3$ x 2); δ_C (100 MHz, $CDCl_3$) 102.6 (C-2), 76.1 (C-3), 64.7 (C-5), 33.5 (C-4), 25.7 ($SiC(\underline{C}H_3)_3$), 25.1 (CH_3), 18.0 ($Si\underline{C}(CH_3)_3$), -4.7 ($SiCH_3$), -5.1 ($SiCH_3$); Found (ESI): 215.1469 $[M+H-H_2O]^+$, ($C_{11}H_{23}O_2Si$ requires 215.1462). Data reported for the major product.

(*S*)-3-(*tert*-Butyldimethylsilyloxy)-4-methylpent-4-en-1-ol 99

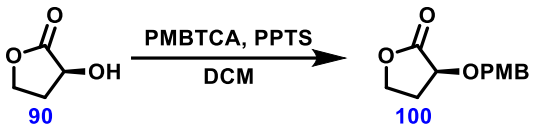


98 99

Lactol **98** (112 mg, 0.50 mmol) was dissolved in anhydrous THF (2 mL) and Petasis reagent (7.14 mL, 1.5 mmol, 0.21 M) was added under N_2. The reaction mixture was stirred in a sealed boiling tube at 75 °C for 16 h before being diluted with petrol (10 mL) and filtered through

celite. The filtrate was washed with 2 N HCl (10 mL) followed by brine (*ca.* 10 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give an orange oil which was purified by column chromatography (20% EtOAc in petrol) to give alcohol **99** as a pale orange oil (67 mg, 58%); ν_{max} (neat)/cm^{-1} 3430 (OH), 2955, 2930, 2858, 2251, 1471, 1256, 1063; $[\alpha]_D^{25}$= -12 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.98 (1 H, s, 5-*H*H), 4.84 (1 H, s, 5-H*H*), 4.29 (1 H, t, *J* 5.5, 3-H), 3.73 (2 H, m, 1-H_2), 2.36 (1 H, t, *J* 5.5, OH), 1.79 (2 H, q, *J* 6.5, 5.5, 2-H_2), 1.69 (3 H, s, 4-CH_3), 0.91 (9 H, s, $SiC(CH_3)_3$), 0.08 (3 H, s, $SiCH_3$), 0.03 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 147.0 (C-4), 111.2 (C-5), 75.9 (C-3), 60.5 (C-1), 37.7 (C-2), 26.0 ($SiC(\underline{C}H_3)_3$), 18.3 ($Si\underline{C}(CH_3)_3$), 18.1 (4-CH_3), -4.6 ($SiCH_3$), -5.2 ($SiCH_3$). Found (ESI): 253.1595 $[M+Na]^+$, ($C_{12}H_{26}NaO_2Si$ requires 253.1594).

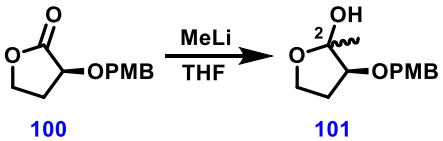
(*S*)-3-((4-Methoxybenzyl)oxy)dihydrofuran-2(3H)-one 100



90 100

Alcohol **90** (200 mg, 1.96 mmol) was dissolved in anhydrous DCM (10 mL) and PMBTCA (1.11 g, 3.92 mmol) and PPTS (50 mg, 0.20 mmol) were added at RT under N_2. The reaction was stirred for 16 h before addition of solid $NaHCO_3$ (*ca*. 2.00 g) and petroleum ether (*ca*. 20 mL). The precipitate was filtered, and the filtrate was concentrated *in vacuo* to give **100** as a colourless oil (435 mg, quant.); δ_H (400 MHz, $CDCl_3$) 7.32 (2 H, d, *J* 8.5, Ar-H), 6.90 (2 H, d, *J* 8.5, Ar-H), 4.87 (1 H, d, *J* 11.5, OC*H*H), 4.67 (1 H, d, *J* 11.5, OC*H*H), 4.41 (1 H, ddd, *J* 9.0, 8.0, 4.5, 5-*H*H), 4.21 (1 H, ddd, *J* 9.0, 8.0, 7.0, 5-*H*H), 4.15 (1 H, t, *J* 7.5, 3-H), 3.81 (3 H, s, OMe), 2.43 (1 H, dddd, *J* 13.0, 7.5, 7.0, 4.5, 4-*H*H), 2.26 (1 H, dq, *J* 13.0, 7.5, 4-*H*H); δ_C (100 MHz, $CDCl_3$) 175.4 (C-2), 159.7 (Ar-C), 130.51 (Ar-C), 129.1 (Ar-C), 114.8 (Ar-C), 72.1 (OCH_2), 72.0 (C-3), 65.7 (C-5), 55.5 (OMe), 30.1 (C-4). Data in accordance with the literature.[212]

(3*S*)-3-(4-Methoxybenzyloxy)-2-methyltetrahydrofuran-2-ol 101

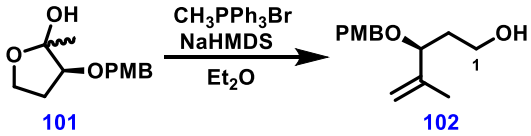


100 101

Lactone **100** (435 mg, 1.96 mmol) was dissolved in anhydrous THF (15 mL) at -78 °C under N_2. MeLi (1.6 M in Et_2O, 1.35 mL, 2.16 mmol) was added and the reaction mixture was stirred

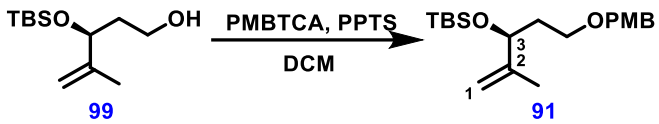
for 3 h at -78 °C followed by addition of sat. aq. NH_4Cl (10 mL). The reaction mixture was extracted with EtOAc (3 x 20 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale yellow oil. This was purified by column chromatography (10% EtOAc in petrol) to give lactol **101** as a colourless oil (320 g, 75%); ν_{max} (neat)/cm^{-1} 3434, 2961, 2929, 2864, 1252, 1103, 1088, 1054, 1021, 1005; δ_H (400 MHz, $CDCl_3$) 7.27 (2 H, m, Ar-H), 6.90 (2 H, m, Ar-H), 4.58 (1 H, m, OCH_2), 4.39 (1 H, m, 5-*H*H), 4.00 (1 H, m, 5-*H*H), 3.98 (1 H, m, 3-H), 3.81 (3 H, s, OMe), 3.75 (2 H, m, 4-H_2), 2.20 (3 H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 159.7 (Ar-H), 129.4 (Ar-H), 114.1 (Ar-H), 113.8 (Ar-H), 102.4 (C-2), 81.6 (C-3), 72.2 (OCH_2), 59.7 (C-4), 55.2 (OMe), 25.3 (CH_3); Found (ESI): 222.1247 $[M+H-H_2O]^+$, ($C_{13}H_{18}O_3$ requires 222.1250). Data reported for the major product.

(*S*)-3-(4-Methoxybenzyloxy)-4-methylpent-4-en-1-ol 102



CH_3PPh_3Br (306 mg, 0.86 mmol) was suspended in anhydrous THF (10 mL) under N_2 and cooled to -78 °C. NaHMDS (1.0 M in THF, 0.86 mL, 0.86 mmol) was added and stirred at this temperature for 1 h. Lactol **101** (160 mg, 0.71 mmol) was added and the reaction mixture stirred at reflux for 24 h. The reaction mixture was quenched by addition of aqueous NH_4Cl (10 mL) and allowed to cool before extraction with Et_2O (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil which was purified by column chromatography (5% EtOAc in petrol) to give alcohol **102** as a colourless oil (48 mg, 28%); $[\alpha]_D^{25}$ = -10 (*c* 1.0, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3347 (OH), 3063, 2914, 2930, 1437, 1182, 1119; δ_H (400 MHz, $CDCl_3$) 7.24 (2 H, d, *J* 8.5, Ar-H), 6.88 (2 H, d, *J* 8.5, Ar-H), 4.99 (2 H, s, 5-H_2), 4.47 (1 H, d, *J* 11.5, OC*H*H), 4.21 (1 H, d, *J* 11.5, OC*H*H), 3.97 (1 H, dd, *J* 9.0, 4.0, 3-H), 3.81 (3 H, s, OMe), 3.73 (2 H, dd, *J* 7.0, 4.5, 1-H_2), 2.40 (1 H, brs, OH), 1.94 (1 H, dddd, *J* 14.5, 9.0, 7.0, 5.0, 2-*H*H), 1.73 (3 H, t, *J* 1.0, 4-CH_3), 1.69 (1 H, m, 2-*H*H); δ_C (100 MHz, $CDCl_3$) 159.4 (Ar-C), 144.4 (Ar-C), 130.4 (Ar-C), 129.6 (C-4), 114.0 (Ar-C), 113.7 (C-5), 82.5 (C-3), 69.9 (OCH_2), 61.4 (C-1), 55.4 (OMe), 36.4 (C-2), 17.1 (CH_3); Found (ESI): 259.1296 $[M+Na]^+$, ($C_{14}H_{20}NaO_3$ requires 259.1305).

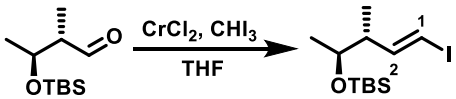
5-(*para*-Methoxybenzyloxy)-(*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylpent-1-ene 91



99 91

Alcohol **99** (1.00 g, 4.34 mmol) and PMBTCA (3.70 g, 13.0 mmol) were dissolved in DCM (30 mL) under N_2. PPTS (108 mg, 0.43 mmol) was added and the reaction mixture stirred at RT for 16 h, after which time solid $NaHCO_3$ (*ca.* 1.00 g) and petroleum ether (50 mL) were added. The reaction mixture was filtered and the solvent removed *in vacuo* to give an orange oil which was purified by column chromatography (2% EtOAc in petrol) to give PMB ether **91** as a colourless oil (600 mg, 39%); ν_{max} (neat)/cm^{-1} 2955, 2928, 2857, 2253, 1513, 1463, 1248, 1086; $[\alpha]_D^{25}$= -11 (*c* 2.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.26 (2 H, d, *J* 8.5, Ar-H), 6.88 (2 H, d, *J* 8.5, Ar-H), 4.87 (1 H, s, 1-H), 4.73 (1 H, s, 1-H), 4.44 (1 H, d, *J* 11.5, OC*H*H), 4.38 (1 H, d, *J* 11.5, OCH*H*), 4.21 (1 H, m, 3-H), 3.81 (3 H, s, OMe), 3.48 (2 H, m, 5-H_2), 1.77 (2 H, m, 4-H_2), 1.68 (3 H, s, 2-CH_3), 0.88 (9 H, s, $SiC(CH_3)_3$), 0.04 (3 H, s, $SiCH_3$), 0.00 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 159.1 (Ar-C), 147.7 (C-2), 130.7 (Ar-C), 129.3 (Ar-C), 113.7 (Ar-C), 110.6 (C-1), 73.6 (C-3), 72.7 (OCH_2), 66.8 (C-5), 55.3 (OMe), 36.5 (C-4), 25.8 ($SiC(\underline{C}H_3)_3$), 18.2 ($Si\underline{C}(CH_3)_3$), 17.1 (2-CH_3), -4.7 ($SiCH_3$), -5.2 ($SiCH_3$); Found (ESI): 373.2176 $[M+Na]^+$, ($C_{20}H_{34}NaO_3Si$ requires 373.2169).

4*S*-(*tert*-butyldimethylsilyloxy)-3*R*,*E*-1-iodo-3-methylpent-1-ene 108

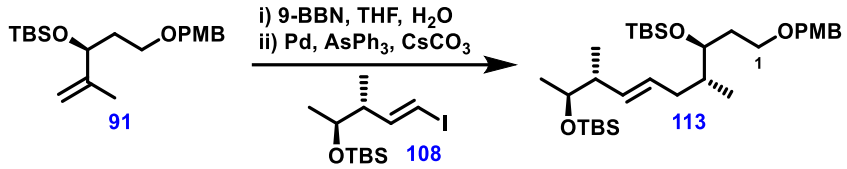


77 108

Anhydrous THF (10 mL) was vigorously stirred, to which was added $CrCl_2$ (3.58 g, 29.3 mmol) and CHI_3 (2.55 g, 6.48 mmol) portionwise over 10 mins at 0 °C under N_2. Aldehyde **77** (700 mg, 3.24 mmol) in anhydrous THF (16 mL) was added dropwise. The resulting solution was stirred at 0 °C for 20 mins, followed by 2 h at RT, before being quenched by addition of saturated aqueous $Na_2S_2O_3$ (30 mL) and stirred for 30 mins. The reaction mixture was extracted with Et_2O (3 x 25 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a yellow oil. This was purified by column chromatography (100% petrol) to give vinyl iodide **108** as a pale yellow oil (350 mg, 32%); ν_{max} (neat)/cm^{-1}

2955, 2928, 2856, 2462, 1372, 1250; $[\alpha]_D^{25}$= +17 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 6.46 (1 H, dd, *J* 14.5, 8.5, 2-H), 5.97 (1 H, dd, *J* 14.5, 1.0, 1-H), 3.64 (1 H, qd, *J* 6.0, 5.0, 4-H), 2.17 (1 H, m, 3-H), 1.07 (3 H, d, *J* 6.0, 5-H_3), 0.98 (3 H, d, *J* 7.0, 3-CH_3), 0.89 (9 H, s, $Si(CH_3)_3$), -0.04 (6 H, s, 2 x $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 149.2 (C-2), 74.9 (C-1), 71.4 (C-4), 48.5 (C-3), 26.0 ($SiC(\underline{C}H_3)_3$), 21.4 (C-5), 18.2 ($Si\underline{C}(CH_3)_3$), 15.9 (3-CH_3), -4.2 ($SiCH_3$), -4.6 ($SiCH_3$); Found (ESI): 363.0608 $[M+Na]^+$, ($C_{12}H_{25}INaOSi$ requires 363.0612).

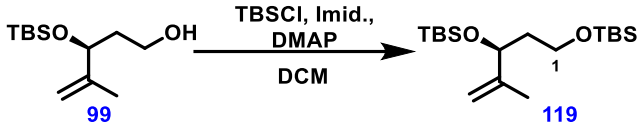
(3*S*,4*R*,8*R*,9*S*,*E*)-1-(*para*-Methoxybenzyloxy)-3,9-bis(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-ene 113



To a stirred solution of alkene **91** (431 mg, 1.23 mmol) in degassed anhydrous THF (4 mL) at -78 °C under N_2 was added a solution of 9-BBN in THF (0.5 M, 7.40 mL, 3.70 mmol). The resulting solution was stirred at RT for 14 h, before being quenched with degassed H_2O (4 mL) and stirred for 1 h. In a separate flask, vinyl iodide **108** (350 mg, 1.03 mmol) was dissolved in degassed DMF (29 mL) and Cs_2CO_3 (1.20 g, 3.70 mmol), $Pd(dppf)Cl_2$ (183 mg, 0.25 mmol) and $AsPh_3$ (76.6 mg, 0.25 mmol) were added and the reaction mixture stirred for 10 mins under N_2 at RT. The borane solution was added dropwise to this mixture and the reaction stirred at RT for 8 h, before being quenched by addition of H_2O (*ca*. 15 mL). The mixture was filtered through celite and extracted with Et_2O (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a brown oil. This was purified by column chromatography (100% petrol) to give alkene **113** as a colourless oil (326 mg, 56%); $[\alpha]_D^{25}$= -20 (*c* 0.5, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2957, 2931, 2856, 1248, 1086, 1067, 1005; δ_H (500 MHz, $CDCl_3$) 7.25 (2 H, d, *J* 8.5, Ar-H), 6.87 (2 H, d, *J* 8.5, Ar-H), 5.33 (2 H, m, 6-H & 7-H), 4.44 (1 H, d, *J* 11.5, OC*H*H), 4.40 (1 H, d, *J* 11.5, OC*H*H), 3.80 (3 H, s, OMe), 3.72 (1 H, m, 3-H), 3.67 (1 H, m, 9-H), 3.51 (1 H, m, 1-*H*H), 3.48 (1 H, m, 1-*H*H), 2.10 (1 H, m, 8-H), 2.00 (1 H, m, 5-*H*H), 1.78 (1 H, m, 5-*H*H), 1.69 (2 H, m, 2-H_2), 1.63 (1 H, m, 4-H), 1.02 (3 H, d, *J* 6.5, 10-H_3), 0.94 (3 H, d, *J* 6.5, 8-CH_3), 0.88 (9 H, s, $SiC(CH_3)_3$), 0.87 (9 H, s, $SiC(CH_3)_3$), 0.85 (3 H, d *J* 6.5, 4-CH_3), 0.03 (6 H, s, $SiCH_3$ x2), 0.01 ($SiCH_3$ x2); δ_C (125 MHz, $CDCl_3$) 159.2 (Ar-C), 134.1 (C-7), 130.9 (Ar-C), 129.4 (Ar-C), 128.9 (C-6), 113.9 (Ar-C), 72.7 (OCH_2), 72.4 (C-

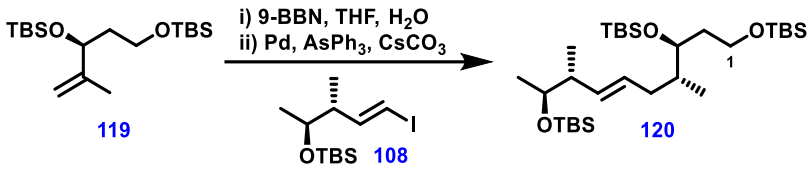
3), 72.1 (C-9), 67.6 (C-1), 55.4 (OMe), 44.4 (C-8), 39.4 (C-4), 36.5 (C-5), 32.0 (C-2), 26.1 (SiC($\underline{C}H_3)_3$), 26.0 (SiC($\underline{C}H_3)_3$), 20.6 (10-CH_3), 18.3 (Si$\underline{C}(CH_3)_3$), 18.2 (Si$\underline{C}(CH_3)_3$), 16.0 (8-CH_3), 14.3 (4-CH_3), -4.2 ($SiCH_3$), -4.2 ($SiCH_3$), -4.5 ($SiCH_3$), -4.7 ($SiCH_3$); Found (MALDI): 587.3928 $[M+Na]^+$, ($C_{32}H_{60}NaO_4Si_2$ requires 587.3928).

(*S*)-1,3-Bis(*tert*-butyldimethylsilyloxy)-4-methylpent-4-ene 119



Alcohol **99** (200 mg, 0.77 mmol) was dissolved in anhydrous DCM (5 mL) and TBSCl (142 mg, 1.16 mmol), imidazole (206 mg, 1.37 mmol) and DMAP (5 mg, 0.08 mmol) were added under N_2. The reaction mixture was stirred at RT for 16 h before the addition of H_2O (5 mL). The aqueous layer was extracted with DCM (3 x 10 mL), the combined organic extracts were dried ($MgSO_4$), and the solvent removed *in vacuo* to give a pale yellow oil. This was purified by filtration through a pad of silica and washing with EtOAc (*ca.* 40 mL). The solvent was removed *in vacuo* to give silyl ether **119** as a colourless oil (268 mg, 94%); ν_{max} (neat)/cm^{-1} 2954, 2929, 2857, 1471, 1361, 1253, 1085; $[\alpha]_D^{25}$= -14 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.86 (1 H, s, 5-*H*H), 4.75 (1 H, s, 5-*H*H), 4.21 (1 H, dd, *J* 7.5, 5.0, 3-H), 3.63 (2 H, m, 1-H_2), 1.73-1.64 (2 H, m, 2-H_2), 1.68 (3 H, s, 4-CH_3), 0.89 (9 H, s, SiC($CH_3)_3$), 0.89 (9 H, s, SiC($CH_3)_3$), 0.04 (3 H, s, $SiCH_3$), 0.04 (6 H, s, $SiCH_3$ x 2), 0.01 (3 H, s, $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 148.0 (C-4), 110.6 (C-5), 73.6 (C-3), 59.9 (C-1), 39.7 (C-2), 26.1 (SiC($\underline{C}H_3)_3$), 26.0 (SiC($\underline{C}H_3)_3$), 18.4 (Si$\underline{C}(CH_3)_3$), 18.4 (Si$\underline{C}(CH_3)_3$), -4.6 ($SiCH_3$), -5.0 ($SiCH_3$), -5.1 ($SiCH_3$), -5.1 ($SiCH_3$); Found (MALDI): 366.2456 $[M+Na]^+$, ($C_{18}H_{40}NaO_2Si_2$ requires 366.2465).

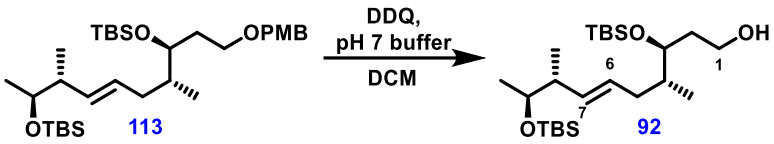
(3*S*,4*R*,8*R*,9*S*,*E*)-1,3,9-Tris(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-ene 120



To a stirred solution of alkene **119** (150 mg, 0.40 mmol) in degassed anhydrous THF (1.5 mL) at -78 °C under N_2 was added a solution of 9-BBN in THF (0.5 M, 2.40 mL, 1.20 mmol). The resulting solution was stirred at RT for 14 h, before being quenched with degassed H_2O (1.5

mL) and stirred for 1 h. In a separate flask, vinyl iodide **108** (115 mg, 0.34 mmol) was dissolved in degassed DMF (9 mL) and Cs_2CO_3 (397 mg, 1.22 mmol), $Pd(dppf)Cl_2$ (66 mg, 0.09 mmol) and $AsPh_3$ (26 mg, 0.09 mmol) were added and stirred for 10 mins under N_2 at RT. The borane solution was added dropwise to this mixture and the reaction stirred at RT for 8 h, before being quenched by addition of H_2O (10 mL). The mixture was filtered through celite and extracted with Et_2O (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a brown oil. This was purified by column chromatography (100% petrol) to give silyl ether **120** as a colourless oil (168 mg, 89%); ν_{max} (neat)/cm^{-1} 2955, 2928, 2856, 1471, 1462, 1252, 1088; $[\alpha]_D^{25}$= -2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.35 (2 H, m, 6-H + 7-H), 3.73-3.68 (3 H, m, 9-H + 1-H_2), 3.63 (1 H, dt, *J* 10.0, 7.5, 3-H), 2.13 (1 H, qd, *J* 6.5, 4.0, 8-H), 2.02 (1 H, ddd, *J* 11.0, 9.0, 5.5, 5-*H*H), 1.79 (1 H, m, 5-*H*H), 1.63 (1 H, m, 4-H), 1.58 (2 H, m, 2-H_2), 1.03 (3 H, d, *J* 6.5, 10-H_3), 0.96 (3 H, d, *J* 6.5, 8-CH_3), 0.90 (9 H, s, $SiC(CH_3)_3$, 0.89 (18 H, s, $SiC(CH_3)_3$ x2), 0.86 (3 H, d, *J* 6.5, 4-CH_3), 0.05 (6 H, s, $SiCH_3$ x2), 0.04 (12 H, s, $SiCH_3$ x4); δ_C (100 MHz, $CDCl_3$) 134.0 (C-7), 129.1 (C-6), 72.2 (C-3), 72.1 (C-9), 60.7 (C-1), 44.4 (C-8), 39.6 (C-4), 36.4 (C-5), 36.2 (C-2), 26.2 ($SiC(\underline{C}H_3)_3$), 26.1 ($SiC(\underline{C}H_3)_3$), 26.1 ($SiC(\underline{C}H_3)_3$), 20.6 (C-10), 18.5 ($Si\underline{C}(CH_3)_3$), 18.3 ($Si\underline{C}(CH_3)_3$), 18.3 ($Si\underline{C}(CH_3)_3$), 16.1 (8-CH_3), 14.4 (4-CH_3), -4.2 ($SiCH_3$), -4.2 ($SiCH_3$), -4.4 ($SiCH_3$), -4.6 ($SiCH_3$), -5.1 ($SiCH_3$), -5.1 ($SiCH_3$). Found (MALDI): 580.4211 $[M+Na]^+$, ($C_{30}H_{66}NaO_3Si_3$ requires 580.4217).

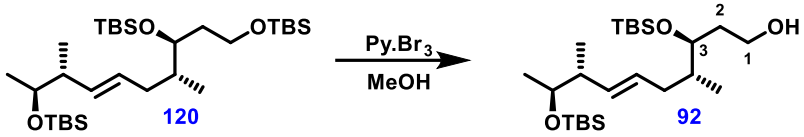
(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-Bis(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-en-1-ol 92



PMB ether **113** (100 mg, 0.18 mmol) was dissolved in DCM (2 mL) and phosphate buffer (2 mL) at 0 °C. DDQ (80 mg, 0.35 mmol) was added and the reaction stirred for 5 h at RT before addition of solid $NaHCO_3$ (100 mg). The reaction mixture was concentrated *in vacuo* and the resulting orange residue purified by column chromatography (5% EtOAc in petrol) to give alcohol **92** as a colourless oil (41 mg, 50%); ν_{max} (neat)/cm^{-1} 3789, 2953, 2929, 2836, 1251, 1084; $[\alpha]_D^{25}$= -7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.33 (2 H, m, 6-H + 7-H), 3.79 (1 H, dt, *J* 8.5, 4.0, 3-H), 3.74 (2 H, m, 1-H_2), 3.68 (1 H, m, 9-H), 2.16 (1 H, m, OH), 2.13 (1 H, td, *J* 7.0, 4.0, 8-H), 2.01 (1 H, m, 5-*H*H), 1.79 (1 H, ddd, *J* 13.5, 8.5, 5.5, 5-*H*H), 1.70 (1 H, m, 4-H), 1.66 (2 H, m, 2-H_2), 1.03 (3 H, d, *J* 6.5, 10-CH_3), 0.96 (3 H, d, *J* 6.5, 8-CH_3), 0.90 (9 H, s, $SiC(CH_3)$,

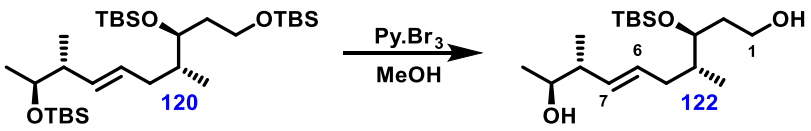
0.88 (9 H, s, SiC(CH_3), 0.86 (3 H, d, J 6.5, 4-CH_3), 0.09 (3 H, s, SiCH_3), 0.07 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3); δ_C (100 MHz, $CDCl_3$) 133.1 (C-7), 128.5 (C-6), 74.7 (C-3), 72.1 (C-9), 61.1 (C-1), 44.4 (C-8), 39.1 (C-4), 36.9 (C-5), 33.2 (C-2), 26.0 (SiC($\underline{C}H_3$)$_3$ x 2), 20.7 (C-10), 18.3 (Si$\underline{C}$(CH_3)), 18.2 (Si$\underline{C}$(CH_3)), 16.2 (8-CH_3), 14.0 (4-CH_3), -4.2 (SiCH_3), -4.2 (SiCH_3), -4.4 (SiCH_3), -4.7 (SiCH_3); Found (MALDI): 467.3353 $[M+Na]^+$, ($C_{24}H_{52}NaO_3Si_2$ requires 467.3358).

(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-Bis(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-en-1-ol 92



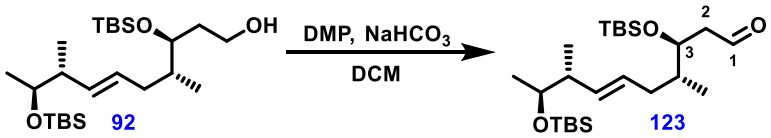
TBS ether **120** (100 mg, 0.18 mmol) was dissolved in anhydrous MeOH (2 mL) and $Py.Br_3$ (2.87 mg, 0.009 mmol) was added at 0 °C under N_2. The reaction mixture was stirred at this temperature for 2.5 h before being quenched with sat. aq. $NaHCO_3$ (*ca.* 5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a yellow oil. This was purified by column chromatography (5% EtOAc in petrol) to give alcohol **92** as a colourless oil (32 mg, 40%); ν_{max} (neat)/cm^{-1} 3789, 2953, 2929, 2836, 1251, 1084; $[\alpha]_D^{25}$= -7 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.33 (2 H, m, 6-H + 7-H), 3.79 (1 H, dt, *J* 8.5, 4.0, 3-H), 3.74 (2 H, m, 1-H_2), 3.68 (1 H, m, 9-H), 2.16 (1 H, m, OH), 2.13 (1 H, m, 8-H), 2.01 (1 H, m, 5-*H*H), 1.79 (1 H, ddd, *J* 13.5, 8.5, 5.5, 5-*H*H), 1.70 (1 H, m, 4-H), 1.66 (2 H, m, 2-H_2), 1.03 (3 H, d, *J* 6.5, 10-H_3), 0.96 (3 H, d, *J* 6.5, 8-CH_3), 0.90 (9 H, s, SiC(CH_3), 0.88 (9 H, s, SiC(CH_3), 0.86 (3 H, d, *J* 6.5, 4-CH_3), 0.09 (3 H, s, SiCH_3), 0.07 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3); δ_C (100 MHz, $CDCl_3$) 133.1 (C-7), 128.5 (C-6), 74.7 (C-3), 72.1 (C-9), 61.1 (C-1), 44.4 (C-8), 39.1 (C-4), 36.9 (C-5), 33.2 (C-2), 26.0 (SiC($\underline{C}H_3$)$_3$ x 2), 20.7 (C-10), 18.3 (Si$\underline{C}$(CH_3)), 18.2 (Si$\underline{C}$(CH_3)), 16.2 (8-CH_3), 14.0 (4-CH_3), -4.2 (SiCH_3), -4.2 (SiCH_3), -4.4 (SiCH_3), -4.7 (SiCH_3); Found (MALDI): 467.3353 $[M+Na]^+$, ($C_{24}H_{52}NaO_3Si_2$ requires 467.3358).

(3*S*,4*R*,8*R*,9*S*,*E*)-3-(*tert*-butyldimethylsilyloxy)-9-hydroxy-4,8-dimethyldec-6-en-1-ol 122



Silyl ether **120** (300 mg, 0.54 mmol) was dissolved in anhydrous MeOH (6 mL) and $Py.Br_3$ (17.2 mg, 0.054 mmol) added at 0 °C under N_2 and stirred for 4 h at this temperature. The reaction was quenched with sat. aq. $NaHCO_3$ (*ca*. 3 mL) and extracted with EtOAc (5 x 10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale orange oil. This was purified column chromatography (15-100% EtOAc in petrol) to give **122** as a colourless oil (134 mg, 75%); ν_{max} (neat)/cm^{-1} 3364 (brs), 2957, 2929, 2866, 1462, 1376, 1253, 1059; $[\alpha]_D^{25}$= + 6 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.48 (1 H, dt, *J* 15.5, 7.0, 6-H), 5.34 (1 H, dd, *J* 15.5, 8.0, 7-H), 3.80 (1 H, m, 3-H), 3.74 (2 H, m, 1-H_2), 3.52 (1 H, m, 9-H), 2.07 (2 H, m, 8 H + 5*H*H), 1.86 (1 H, m, 5-*H*H), 1.73 (1 H, m, 4-H), 1.64 (2 H, m, 2-H_2), 1.17 (3 H, d, *J* 6.5, 10-CH_3), 1.00 (3 H, d, *J* 6.5, 8-CH_3), 0.88 (9 H, s, $SiC(CH_3)_3$), 0.86 (3 H, d, *J* 6.5, 4-CH_3), 0.09 ($SiCH_3$), 0.07 ($SiCH_3$); δ_C (101 MHz, $CDCl_3$) 133.7 (C-7), 131.1 (C-6), 74.0 (C-3), 71.4 (C-9), 60.8 (C-1), 45.1 (C-8), 38.9 (C-4), 36.8 (C-5), 33.5 (C-2), 26.0 ($SiC(\underline{C}H_3)_3$), 16.8 (8-CH_3), 14.2 (4-CH_3), -4.2 ($SiCH_3$), -4.4 ($SiCH_3$); Found (ESI): 353.2467 $[M+Na]^+$, ($C_{18}H_{38}NaO_3Si$ requires 353.2482).

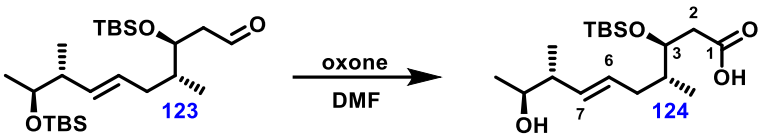
(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-bis(*tert*-Butyldimethylsilyloxy)-4,8-dimethyldec-6-enal 123



Alcohol **92** (130 mg, 0.29 mmol) was dissolved in anhydrous DCM (10 mL), $NaHCO_3$ (121 mg, 1.45 mmol) and the reaction mixture was cooled to 0 °C under N_2. DMP (1.08 mL, 0.38 mmol) was added dropwise and the reaction mixture stirred at RT for 1.5 h, after which time sat. aq. $Na_2S_2O_3$ (*ca.* 5 mL) was added. The reaction mixture was extracted with DCM (3 x 10 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a yellow oily solid. This was triturated with Et_2O (*ca.* 2 mL), filtered and the solvent removed *in vacuo* to give aldehyde **123** as a colourless oil (125 mg, 98%) which was used without further purification; ν_{max} (neat)/cm^{-1} 2929, 2857, 1729, 1462, 1375, 1253, 1087; $[\alpha]_D^{25}$= +2 (*c*

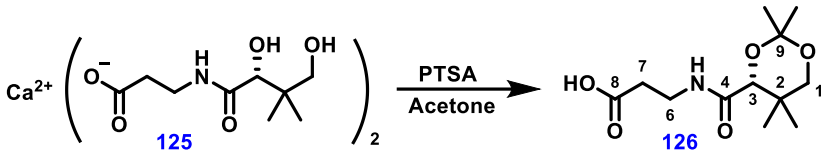
1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 9.79 (1 H, dd, *J* 3.0, 2.0, 1-H), 5.34 (2 H, m, 6-H & 7-H), 4.15 (1 H, dt, *J* 8.0, 4.0, 3-H), 3.68 (1 H, qd, *J* 6.5, 4.0, 9-H), 2.50 (1 H, ddd, *J* 15.5, 8.0, 3.0, 2-*H*H), 2.38 (1 H, ddd, *J* 15.5, 4.0, 2.0, 2-*H*H), 2.11 (1 H, m, 8-H), 2.01 (1 H, m, 5-*H*H), 1.82 (1 H, m, 5-*H*H), 1.71 (1 H, m, 4-H), 1.02 (3 H, d, *J* 6.5, 10-H_3), 0.95 (3 H, d, *J* 6.5, 8-CH_3), 0.87 (21 H, m, 2 x $Si(CH_3)_3$ & 4-CH_3), 0.03 (12 H, m, 4 x $SiCH_3$); δ_C (125 MHz, $CDCl_3$) 202.8 (C-1), 134.8 (C-7), 128.1 (C-6), 72.0 (C-9), 71.1 (C-3), 46.5 (C-2), 44.4 (C-8), 39.7 (C-4), 36.6 (C-5), 26.0 ($C(\underline{C}H_3)_3$), 25.9 ($C(\underline{C}H_3)_3$), 20.8 (C-10), 18.3 ($\underline{C}(CH_3)_3$), 18.2 ($\underline{C}(CH_3)_3$), 16.3 (8-CH_3), 14.2 (4-CH_3), -4.2 ($SiCH_3$), -4.3 ($SiCH_3$), -4.5 ($SiCH_3$), -4.7 ($SiCH_3$); Found (ESI): 443.3373 $[M+H]^+$, ($C_{24}H_{51}O_3Si_2$ requires 443.3371).

(3*S*,4*R*,8*R*,9*S*,*E*)-3-(*tert*-Butyldimethylsilyloxy)-9-hydroxy-4,8-dimethyldec-6-enoic acid 124



Aldehyde **99** (30 mg, 0.07 mmol) was dissolved in anhydrous DMF (0.7 mL) and oxone (20 mg, 0.07 mmol) was added and stirred at RT for 3 h under N_2. HCl (1 M, *ca.* 1 mL) was added and the reaction mixture extracted with EtOAc (3 x 1 mL). The combined organic extracts were washed with water (3 x 5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale yellow oil. This was purified by column chromatography (20-100% EtOAc in petrol) to give acid **100** as a colourless oil (12.5 mg, 52%) ; ν_{max} (neat)/cm^{-1} 3410 (brs), 2959, 2929, 2866, 1712, 1462, 1378, 1252, 1080; $[\alpha]_D^{25}$= +4 (*c* 1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 5.48 (1 H, dt, *J* 14.5, 7.0, 6-H), 5.38 (1 H, ddt, *J* 15.5, 8.5, 1.5, 7-H), 4.09 (1 H, m, 3-H), 3.53 (1 H, p, *J* 6.0, 9-H), 2.43 (2 H, d, *J* 6.0, 2-H_2), 2.08 (2 H, m, 8-H, 5-*H*H), 1.88 (1 H, m, 5-H*H*), 1.73 (1 H, m, 4-H), 1.16 (3 H, d, *J* 6.5, 10-H_3), 1.00 (3 H, d, *J* 6.5, 8-CH_3), 0.88 (3 H, d, *J* 6.5, 4-CH_3), 0.87 (9 H, s, $SiC(CH_3)_3$, 0.07 ($SiCH_3$), 0.06 (3 H, s, $SiCH_3$) δ_C (125 MHz, $CDCl_3$) 176.4 (C-1), 133.9 (C-7), 130.4 (C-6), 72.2 (C-3), 71.2 (C-9), 44.9 (C-8), 39.0 (C-4), 37.6 (C-2), 36.2 (C-5), 25.8 ($SiC(\underline{C}H_3)_3$), 20.2 (C-10), 18.0 ($Si\underline{C}(CH_3)_3$), 16.6 (8-CH_3), 14.3 (4-CH_3), -4.6 ($SiCH_3$), -4.8 ($SiCH_3$); Found (ESI): 343.2313 $[M-H]^-$, ($C_{18}H_{35}O_4Si$ requires 343.2305).

(*R*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propionic acid 126



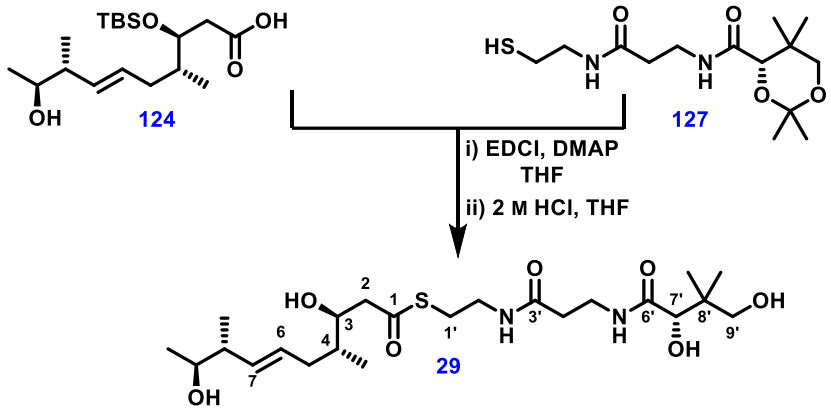
Acetone (50 mL) was stirred with molecular sieves for 20 mins before calcium D-pantothenate **125** (1.0 g, 2.09 mmol) and PTSA (0.96 g, 5.56 mmol) were added under N_2. The reaction mixture was stirred at RT for 16 h before being filtered through celite and washed with acetone (30 mL). The solvent was removed *in vacuo* to give a white residue which was dissolved in EtOAc (20 mL) and washed with brine (20 mL). The organic extract was dried ($MgSO_4$) and the solvent removed *in vacuo* to give a white solid which was triturated with n-hexanes (*ca*. 5 mL) to give **126** as a white solid (444 mg, 82%); $[\alpha]_D^{25}$= +62.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.01 (1 H, m, NH), 4.10 (1 H, s, 3-H), 3.70 (1 H, d, *J* 12.0, 1-*H*H), 3.58 (1 H, m, 6-*H*H), 3.49 (1 H, m, 6-H*H*), 3.30 (1 H, d, *J* 12.0, 1-*H*H), 2.69 (2 H, t, *J* 6.0, 7-H_2), 1.46 (3 H, s, 9-CH_3), 1.43 (3 H, s, 9-CH_3), 1.04 (3 H, s, 2-CH_3), 0.98 (3 H, s, 2-CH_3); δ_C (100 MHz, $CDCl_3$) 176.5 (C-8), 170.3 (C-4), 99.2 (C-9), 77.1 (C-3), 71.5 (C-1), 34.2 (C-6), 33.9 (C-7), 33.0 (C-2), 29.5 (9-CH_3), 22.1 (2-CH_3), 18.9 (2-CH_3), 18.8 (9-CH_3). Data in accordance with the literature. [213]

Pantetheine dimethyl ketal 127

Acid **126** (410 mg, 1.58 mmol) was dissolved in anhydrous THF (8 mL) under N_2 and CDI (374 mg, 2.30 mmol) was added. The reaction mixture was stirred at RT for 30 mins before cysteamine HCl (262 mg, 3.40 mmol) was added and the reaction mixture stirred for 16 h. The reaction was quenched by addition of sat. aq. NH_4Cl (10 mL) and extracted with DCM (3 x 10 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give a colourless oil. This was purified by column chromatography (100% EtOAc) to give **127** as a white solid (377 mg, 75%); m.p 99-101 °C; $[\alpha]_D^{25}$= +33.4 (*c* 1.0, MeOH); δ_H (400 MHz, $CDCl_3$) 6.90 (1 H, br s, NH), 6.21 (1 H, br s, NH), 4.08 (1 H, s, 3-H), 3.70 (1 H, d, *J* 12.0, 1-*H*H), 3.57 (2 H, m, 6-H_2), 3.47 (2 H, m, 10-H_2), 3.27 (1 H, d,

J 12.0, 1-*H*H), 2.66 (2 H, q, *J* 7.0, 11-H_2), 2.47 (2 H, t, *J* 6.0, 7-H_2), 1.46 (3 H, s, 12-CH_3), 1.42 (3 H, s, 12-CH_3), 1.36 (1 H, t, *J* 8.5, SH), 1.09 (3 H, s, 2-CH_3), 0.97 (3 H, s, 2-CH_3); δ_C (100 MHz, $CDCl_3$) 171.1 (C-8), 170.2 (C-4), 99.1 (C-12), 77.1 (C-3), 71.4 (C-1), 42.4 (C-10), 36.1 (C-7), 34.8 (C-6), 32.9 (C-2), 29.5 (12-CH_3), 22.1 (2-CH_3), 18.9 (2-CH_3), 18.7 (12-CH_3). Data in accordance with the literature. [214]

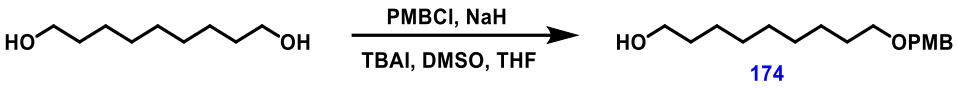
(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-dihydroxy-4,8-dimethyldec-6-enethioate-pantetheine 29



Acid **124** (9.5 mg, 0.03 mmol) was dissolved in anhydrous DCM (0.2 mL) under N_2. Protected pantetheine **127** (29 mg, 0.09 mmol) and DMAP (0.4 mg, 0.003 mmol) were added. The reaction mixture was cooled to 0 °C and EDCI (7.2 mg, 0.038 mmol) was added. The reaction was stirred at this temperature for 5 mins followed by RT for 16 h. HCl (1 M, 1 mL) was added and the two layers were separated. The aqueous layer was extracted with EtOAc (3 x 1 mL), the combined organic extracts dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale yellow oil (16.5 mg, 0.03 mmol) which was dissolved in THF (0.5 mL). HCl (2 M, 0.25 mL) was added and the reaction mixture was stirred at RT for 9 h before addition of sat. aq. $NaHCO_3$ (*ca*. 1 mL) and extraction with EtOAc (3 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a colourless oil. This was purified by column chromatography (5-15% MeOH in DCM) to give **29** as a colourless oil (14.7 mg, 98%); ν_{max} (neat)/cm^{-1} 3676 (brs), 3344 (brs), 2972, 2920, 2904, 1708, 1655, 1578, 1451, 1404, 1252, 1069; $[\alpha]_D^{25}$= +4 (*c* 0.5, MeOD); δ_H (500 MHz, MeOD) 5.46 (2 H, m, 6-H + 7-H), 3.97 (1 H, ddd, *J* 9.0, 6.0, 3.0, 3-H), 3.92 (1 H, s, 7'-H), 3.63 (1 H, qd, *J* 6.0, 5.0, 9-H), 3.47 (2 H, m, 5'-H_2), 3.47 (1 H, m, 9'-*H*H), 3.40 (1 H, m, 9'-H*H*), 3.36 (2 H, m, 2'-H_2), 3.03 (2 H, t, *J* 6.5, 1'-H_2), 2.74 (1 H, dd, *J* 15.0, 3.5, 2-*H*H), 2.69 (1 H, m, 2-H*H*), 2.42 (2 H, t, *J* 6.5, 4'-H_2), 2.21 (1 H, m, 5-*H*H), 2.16 (1 H, m, 8-H), 1.91 (1 H, m, 5-*H*H), 1.64 (1 H, m, 4-H), 1.12 (3 H, s, 10-H_3), 1.02 (3 H,

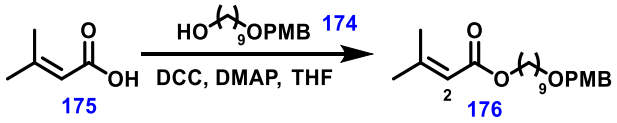
d, *J* 6.5, 8-CH_3), 0.93 (6 H, s, 2 x 8'-CH_3), 0.90 (3 H, d, *J* 6.5, 4-CH_3); δ_C (125 MHz, MeOD) 199.4 (C-1), 176.1 (C-6'), 174.0 (C-3'), 135.3 (C-7), 130.1 (C-6), 77.3 (C-7'), 73.0 (C-3), 72.2 (C-9), 70.4 (C-9'), 49.1 (C-2), 45.3 (C-8), 40.4 (C-4), 40.0 (C-2'), 36.7 (C-5), 36.4 (C-4'), 36.3 (C-5'), 29.3 (C-1'), 21.3 (C-8'), 20.9 (8'-CH_3 x 2), 20.2 (C-10), 16.6 (8-CH_3), 15.6 (4-CH_3); Found (ESI): 513.2572 $[M+Na]^+$,($C_{23}H_{42}N_2NaO_7S$ requires 513.2570).

9-(4-Methoxybenzyloxy)nonan-1-ol 174



To a suspension of NaH (60% in mineral oil, 91 mg, 2.29 mmol) in anhydrous THF/DMSO (1:1) (10 mL) was added 1,9 nonanediol (1.00 g, 6.24 mmol) under N_2 at 0°C. The mixture was stirred at 0 °C for 1 h, after which time TBAI (76.7 mg, 0.21 mmol) and PMBCl (0.28 mL, 2.08 mmol) were added. The mixture was stirred for 16 h at RT before sat. aq. NH_4Cl (15 mL) was added. The reaction mixture was extracted with EtOAc (3 x 20 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil which was purified by column chromatography (10% EtOAc in hexane) to give **174** as a colourless oil (1.48 g, 85%); δ_H (400 MHz, $CDCl_3$) 7.28 (2 H, d, *J* 8.5, Ar-H), 6.90 (2 H, d, *J* 8.5, Ar-H), 4.45 (2 H, s, OCH_2Bn), 3.83 (3 H, s, OMe), 3.66 (2 H, t, *J* 6.5, OCH_2), 3.45 (2 H, t, *J* 6.5, OCH_2), 1.60 (4 H, m, 2 x CH_2), 1.33 (10 H, m, 5 x CH_2 + OH); δ_C (100 MHz, $CDCl_3$) 159.1 (Ar-C), 130.8 (Ar-C), 129.2 (Ar-C), 113.7 (Ar-C), 72.5 ($O\underline{C}H_2Bn$), 70.2 (OCH_2), 63.0 (OCH_2), 55.3 (OMe), 32.8 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 26.2 (CH_2), 25.7 (CH_2). Data in accordance with the literature.[215]

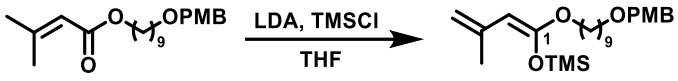
9-(4-Methoxybenzyloxy)nonyl 3-methylbut-2-enoate 176



To a stirred solution of 3-3-dimethylacrylic acid **175** (160 mg, 1.60 mmol), in anhydrous DCM (4 mL) was added DMAP (26 mg, 0.21 mmol), alcohol **174** (300 mg, 1.07 mmol) and DCC (330 mg, 1.60 mmol) at 0°C under N_2. The reaction mixture was stirred for 16 h at RT. After this time, the reaction mixture was filtered through a pad of celite and the solvent removed *in vacuo* to give a colourless oil which was purified by column chromatography (5% EtOAc in

hexane) to give **176** as a colourless oil (487 mg, 84%); ν_{max} (neat)/cm^{-1} 2929, 2854, 1723, 1511, 1152; δ_H (400 MHz, $CDCl_3$) 7.19 (2 H, d, *J* 8.5, Ar-H), 6.80 (2 H, d, *J* 8.5, Ar-H), 5.60 (1 H, s, 2-H), 4.36 (2 H, s, OCH_2Bn), 4.00 (2 H, t, *J* 6.5, OCH_2), 3.73 (3 H, s, OMe), 3.36 (2 H, t, *J* 6.5, OCH_2), 2.20 (3 H, s, 4-H_3), 1.89 (3 H, s, 3-CH_3), 1.66 (4 H, m, 2 x CH_2), 1.35 (10 H, m, 5 x CH_2); δ_c (100 MHz, $CDCl_3$) 166.8 (C-1), 159.1 (Ar-C), 156.4 (C-3), 130.8 (Ar-C), 129.2 (Ar-C), 115.9 (C-2), 113.7 (Ar-C), 72.5 (OCH_2Ar), 72.3 (CH_2OPMB), 63.7 ($CO_2\underline{C}H_2$), 55.3 (OMe), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 27.4 (C-4), 26.2 (CH_2), 26.0 (CH_2), 20.2 (2 x 3-CH_3); Found (ESI): 385.2347 $[M+Na]^+$, ($C_{22}H_{34}O_4Na$ requires 385.2345).

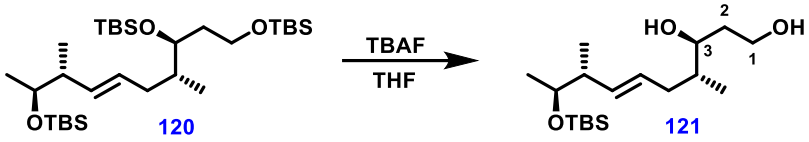
Silyl dienol ether 171



176 171

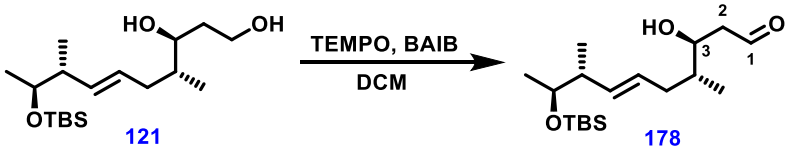
To a stirred solution of DIPA (0.14 mL, 1.02 mmol) in anhydrous THF (5 mL) at -78 °C was added nBuLi (1.49 M in hexanes, 0.68 mL, 1.02 mmol) dropwise under N_2. The reaction mixture was stirred at this temperature for 30 mins after which time PMB ether **176** (300 mg, 0.83 mmol) was added dropwise and stirred for 15 mins followed by the addition of TMSCl (0.105 mL, 0.82 mmol). The reaction mixture was stirred for a further 30 mins at this temperature and then warmed to RT for an additional 1 h. The reaction was diluted with pentane (*ca.* 3 mL) and the supernatant removed by pipette and concentrated *in vacuo*. The crude oil was redissolved in pentane and filtered. The filtrate was concentrated *in vacuo* to give silyl dienol ether **171** as an orange oil which was used without further purification (420 mg, 95%); ν_{max} (neat)/cm^{-1} 2929, 2855, 1644, 1517, 1242, 841; δ_H (400 MHz, $CDCl_3$) 7.26 (2 H, d, *J* 8.5, Ar-H), 6.88 (2 H, d, *J* 8.5, Ar-H), 4.76 (1 H, s, 4-*H*H), 4.52 (1 H, s, 4-*H*H), 4.43 (2 H, s, OCH_2Ar), 4.22 (1 H, s, 2-H), 3.80 (3 H, s, OCH_3), 3.71 (2 H, t, *J* 6.5, CO_2CH_2), 3.43 (2 H, t, *J* 6.5, CH_2OPMB), 1.92 (3 H, s, 3-CH_3), 1.63 (4 H, m, 2 x CH_2), 1.30 (10 H, m, 5 x CH_2), 0.24 (9 H, s, TMS); δ_C (100 MHz, $CDCl_3$) 159.1 (Ar-C), 156.7 (C-3), 140.6 (C-1), 130.8 (Ar-C), 129.3 (Ar-C), 113.7 (Ar-C), 107.0 (C-4), 80.8 (C-2), 72.5 (OCH_2Ar), 70.2 (CH_2OPMB), 67.8 ($CO_2\underline{C}H_2$), 55.3 (OCH_3), 29.8 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 26.2 (CH_2), 26.1 (CH_2), 23.7 (3-CH_3), 0.5 ($Si(CH_3)_3$); Found (ESI): 457.2748 $[M+Na]^+$, ($C_{25}H_{42}NaO_4Si$ requires 457.2745).

(3*S*,4*R*,8*R*,9*S*,*E*)-9-(*tert*-butyldimethylsilyloxy)-3-hydroxy-4,8-dimethyldec-6-en-1-ol 121



To a stirred solution of **120** (150 mg, 0.27 mmol) in anhydrous THF (2.5 mL) under N_2 was added TBAF (2.7 mL, 2.7 mmol) and the reaction mixture stirred at RT for 20 h, followed by addition of sat. aq. NH_4Cl (*ca*. 5 mL) and extraction with EtOAc (3 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (50% EtOAc in petrol) to give **121** as a colourless oil (66 mg, 74%); ν_{max} (neat)/cm^{-1} 3346 (brs), 2957, 2928, 2857, 1462, 1361, 1252, 1031; $[\alpha]_D^{25}$= +4 (*c* 2.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.41 (2 H, m, 7-H + 6-H), 3.85 (2 H, m, 1-H_2), 3.69 (2 H, m, 9-H + 3-H), 2.40 (1 H, brs, OH), 2.37 (1 H, brs, OH), 2.15 (2 H, m, 8-H + 5*H*H), 1.96 (1 H, m, 5-*H*H), 1.70 (2 H, m, 2-H_2), 1.64 (1 H, m, 4-H), 1.04 (3 H, d, *J* 6.5, 10-H_3), 0.96 (3 H, d, *J* 6.5, 8-CH_3), 0.89 (3 H, d, *J* 6.5, 4-CH_3), 0.88 (9 H, s, $SiC(CH_3)_3$), 0.04 (6 H, s, 2 x $SiCH_3$); δ_C (100 MHz, $CDCl_3$) 134.7 (C-7), 128.5 (C-6), 76.7 (C-3), 72.1 (C-9), 62.5 (C-1), 44.4 (C-8), 39.6 (C-4), 36.5 (C-5), 34.8 (C-2), 26.0 ($SiC(\underline{C}H_3)_3$), 20.9 (C-10), 18.3 ($Si\underline{C}(CH_3)_3$), 16.4 (8-CH_3), 15.5 (4-CH_3), -4.2 ($SiCH_3$), -4.7 ($SiCH_3$); Found (ESI): 353.2476 $[M+Na]^+$, ($C_{18}H_{38}NaO_3Si$ requires 353.2482).

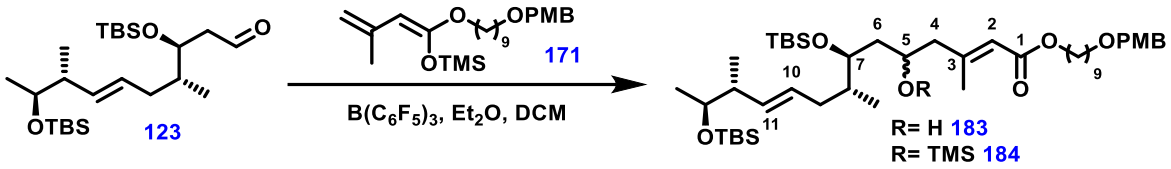
(3*S*,4*R*,8*R*,9*S*,*E*)-9-(*tert*-butyldimethylsilyloxy)-3-hydroxy-4,8-dimethyldec-6-enal 178



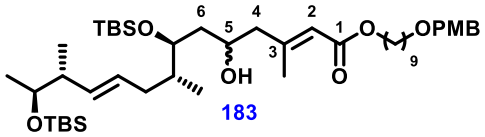
Triol **121** (40 mg, 0.12 mmol) was dissolved in anhydrous DCM (0.5 mL) and TEMPO (3 mg, 0.02 mmol) was added followed by BAIB (39 mg, 0.12 mmol) under N_2. The reaction was stirred at RT for 3 h before addition of sat. aq. $Na_2S_2O_3$ (*ca*. 1 mL) and extraction with DCM (3 x 3 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale orange oil. This was purified by column chromatography (15% EtOAc in petrol) to give **178** as a colourless oil (1.97 mg, 5%); $[\alpha]_D^{25}$= +3 (*c* 1.0, $CHCl_3$); ν_{max} (neat)/cm^{-1} 3457 (brs), 2957, 2928, 2856, 1725, 1471, 1373, 1361, 1252, 1031; δ_H (500 MHz, $CDCl_3$) 9.87 (1 H, s, 1-H), 5.39 (2 H, m, 6-H + 7-H), 4.02 (1 H, dd, *J* 8.5, 6.0, 3-H), 3.68 (1 H, td, *J* 6.0, 4.5, 9-

H), 2.60 (2 H, m, 2-H_2), 2.19 (1 H, m, 5-*H*H), 2.12 (1 H, m, 8-H), 1.89 (1 H, m, 5-*H*H), 1.62 (1 H, m, 4-H), 1.03 (3 H, d, *J* 6.5, 10-H_3), 0.96 (3 H, d, *J* 6.5, 8-CH_3), 0.90 (3 H, d, *J* 6.5, 4-CH_3), 0.88 (9 H, s, SiC(CH_3)$_3$), 0.03 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3); δ_C (125 MHz, $CDCl_3$) 203.1 (C-1), 135.0 (C-7), 128.0 (C-6), 72.1 (C-9), 70.7 (C-3), 47.6 (C-2), 44.4 (C-8), 38.8 (C-4), 36.2 (C-5), 26.0 (SiC($\underline{C}H_3$)$_3$), 20.9 (C-10), 18.3 (Si$\underline{C}$(CH_3)$_3$), 16.4 (8-CH_3), 15.4 (4-CH_3), -4.2 (SiCH_3), -4.7 (SiCH_3); Found (ESI): 351.2318 $[M+Na]^+$, ($C_{18}H_{36}NaO_3Si$ requires 351.2326).

Treatment of aldehyde 123 with silyl dienol ether 171

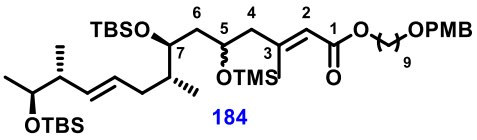


Aldehyde **123** (105 mg, 0.24 mmol) was dissolved in anhydrous DCM:Et_2O 9:1 (2 mL) and cooled to -78 °C. Silyl dienol ether **171** (206 mg, 0.47 mmol) was added followed by $B(C_6F_5)_3$ (123 mg, 0.24 mmol). The reaction was stirred at this temperature for 1 h before concentrating *in vacuo* to give a pale orange oil which was purified by column chromatography (10% EtOAc in petrol) to give **183** and **184** as colourless oils, both isolated as a 1:1 mixture of diastereomers.



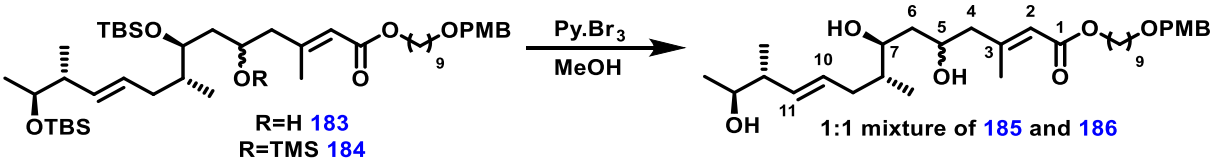
Alcohol **183** was repurified by column chromatography (20% DCM in $CHCl_3$) to give a colourless oil (52.1 mg, 27%); ν_{max} (neat)/cm^{-1} 3464, 2952, 2928, 2855, 1715, 1646, 1513, 1462, 1248, 1146, 1093; δ_H (500 MHz, $CDCl_3$, 1:1 mixture of diastereomers) 7.26 (2 H, d, *J* 8.5, Ar-H), 6.87 (2 H, d, *J* 8.5, Ar-H), 5.73 (1 H, s, 2-H), 5.33 (2 H, m, 10-H & 11-H), 4.43 (2 H, s, OCH_2Ar), 4.12 (1 H, m, 7-H), 4.07 (2 H, t, J 6.5, 1'-H_2), 3.90 (1 H, m, 5-H), 3.80 (3 H, s, OMe), 3.67 (1 H, m, 13-H), 3.43 (2 H, t, *J* 6.5, 9'-H_2), 2.30 (1 H, m, 6-*H*H), 2.23 (1 H, m, 6-*H*H), 2.20 (3 H, d, *J*, 1.0 3-H_3), 2.12 (1 H, m, 12-H), 1.85 (1 H, m, 9-*H*H), 1.73 (2 H, m, 8-H + 9-H*H*), 1.47 (2 H, m, 4-H_2), 1.28 (14 H, m, CH_2 x 7), 1.02 (3 H, d, *J* 6.5, 14-H_3), 0.95 (3 H, d, *J* 6.5, 12-CH_3), 0.90 (9 H, s, SiC(CH_3)$_3$), 0.88 (3 H, d, *J* 6.5, 8-CH_3), 0.88 (9 H, s, SiC(CH_3)$_3$), 0.11 (3 H, s, SiCH_3), 0.10 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3), 0.03 (3 H, s, SiCH_3); δ_C (125 MHz, $CDCl_3$) 166.8 (C-1), 159.2 (Ar-C), 156.4 (C-3), 134.6 (C-11), 131.0 (Ar-C), 129.4 (Ar-C), 128.5 (C-10), 128.2 (Ar-C), 118.2

(C-2), 118.1 (Ar-C), 113.9 (Ar-C). 76.1 (C-5), 72.7 (O<u>C</u>H$_2$Ar), 71.9 (C-13), 70.4 (C-9'), 66.4 (C-7), 64.0 (C-1'), 55.4 (OMe), 49.2 (C-6), 44.6 (C-12), 39.5 (C-8), 37.1 (C-9), 36.5 (C-4), 29.6 (CH_2), 26.3 (CH_2), 26.1 (SiC(<u>C</u>H$_3$)$_3$), 26.0 (SiC(<u>C</u>H$_3$)$_3$), 20.8 (C-14), 18.1 (Si<u>C</u>(CH$_3$)$_3$), 18.0 Si<u>C</u>(CH$_3$)$_3$), 16.3 (12-CH$_3$), 13.4 (8-CH$_3$), -4.2 (SiCH$_3$), -4.3 (SiCH$_3$), -4.5 (SiCH$_3$), -4.7 (SiCH$_3$); Found (MALDI): 827.5659 $[M+Na]^+$,($C_{46}H_{84}NaO_7Si_2$ requires 827.5648).



Silyl ether **184** was repurified by column chromatography (60% DCM in $CHCl_3$) to give a colourless oil (73.6 mg, 35%); ν_{max} (neat)/cm^{-1} 2954, 2928, 2855, 1716, 1513, 1462, 1374, 1248, 1146, 1094; δ_H (500 MHz, $CDCl_3$, 1:1 mixture of diastereomers) 7.26 (2 H, d, *J* 8.5, Ar-H), 6.87 (2 H, d, *J* 8.5, Ar-H), 5.68 (1 H, s, 2-H), 5.36 (2 H, m, 10-H + 11-H), 4.43 (2 H, s, OCH$_2$Ar), 4.08 (2 H, m, 1'-H$_2$), 3.97 (1 H, m, 5-H), 3.80 (3 H, s, OMe), 3.74 (1 H, m, 7-H), 3.69 (1 H, m, 13-H), 3.43 (2 H, t, *J* 6.5, 9'-H$_2$), 2.31 (1 H, m, 6-*H*H), 2.24 (1 H, m, 6-*H*H), 2.16 (3 H, d, *J* 1.0, 3-CH$_3$), 2.10 (1 H, m, 12-H), 1.95 (1 H, m, 9-*H*H), 1.80 (1 H, m, 9-*H*H), 1.64 (1 H, m, 8-H), 1.53 (1 H, m, 4-*H*H), 1.43 (1 H, m, 4-H*H*), 1.03 (3 H, d, *J* 6.5, 14-H$_3$), 0.95 (3 H, d, *J* 6.5, 12-CH$_3$), 0.88 (3 H, d, J 6.5, 8-CH$_3$), 0.88 (18 H, s, 2 x SiC(CH$_3$)$_3$), 0.11 (9 H, s, TMS), 0.06 (3 H, s, SiCH$_3$), 0.05 (3 H, s, SiCH$_3$), 0.04 (3 H, s, SiCH$_3$), 0.03 (3 H, s, SiCH$_3$); δ_C (125 MHz, $CDCl_3$) 166.8 (C-1), 159.2 (Ar-C), 156.8 (C-3), 134.0 (C-11), 131.0 (Ar-C), 129.4 (Ar-C), 128.9 (C-10), 118.5 (C-2), 118.3 (Ar-C), 113.9 (Ar-C), 73.0 (C-7), 72.7 (O<u>C</u>H$_2$Ar), 72.2 (C-13), 70.4 (C-9'), 69.0 (C-5), 63.9 (C-1'), 55.4 (OMe), 50.3 (C-6), 44.4 (C-12), 40.6 (C-4), 39.1 (C-8), 36.1 (C-9), 29.6 (CH_2), 29.4 (CH_2), 28.9 (CH_2), 26.3 (CH_2), 26.1 (SiC(<u>C</u>H$_3$)$_3$), 26.0 (SiC(<u>C</u>H$_3$)$_3$), 20.7 (C-14), 19.4 (3-CH$_3$), 18.3 (Si<u>C</u>(CH$_3$)$_3$), 18.2 (Si<u>C</u>(CH$_3$)$_3$), 14.3 (12-CH$_3$), 0.97 (TMS), -4.1 (SiCH$_3$), -4.1 (SiCH$_3$), -4.2 (SiCH$_3$), -4.6 (SiCH$_3$); Found (MALDI): 899.6049 $[M+Na]^+$,($C_{49}H_{92}NaO_7Si_3$ requires 899.6043).

Treatment of silyl ethers 183 and 184 with Py.Br$_3$

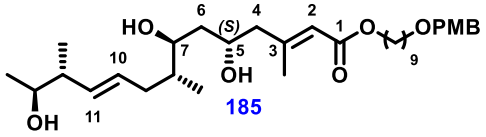


Silyl ether **184** (74 mg, 0.08 mmol) was dissolved in anhydrous MeOH (1 mL) under N_2 and Py.Br$_3$ (8.9 mg, 0.03 mmol) added. The reaction was stirred at RT for 5 h before addition of

sat. aq. $NaHCO_3$ (*ca*. 2 mL). The mixture was extracted with Et_2O (3 x 3 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a pale orange oil which was purified by column chromatography (70% EtOAc in petrol) to give alcohols **185** and **186** as a colourless oil and a 1:1 mixture of diastereomers (41.4 mg, 85%).

The above reaction was repeated using alcohol **183** (52 mg, 0.06 mmol) to give alcohols **185** and **186** as a 1:1 mixture (20.2 mg, 82%). The product from both of these reactions were combined and the diastereomers separated by column chromatography (100% EtOAc) to give alcohol **186** (35 mg) and alcohol **185** (25 mg).

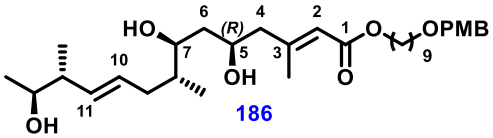
***para*-Methoxybenzyl ether of (*S*)-des-6-hydroxy-desepoxy mupirocin W4-OH 185**



185

ν_{max} (neat)/cm^{-1} 3327, 2950, 2929, 2850, 1769, 1642, 1513, 1462, 1245, 1145, 1093; $[\alpha]_D^{25}$= +3 (*c* 1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.25 (2 H, d, *J* 8.5, Ar-H), 6.87 (2 H, d, *J* 8.5, Ar-H), 5.73 (1 H, s, 2-H), 5.53 (1 H, dt, *J* 14.5, 7.0, 11-H), 5.33 (1 H, m, 10-H), 4.42 (2 H, s, OCH_2Ar), 4.06 (2 H, t, *J* 6.5, 1'-CH_2), 4.03 (1 H, m, 5-H), 3.80 (3 H, s, OMe), 3.71 (1 H, m, 7-H), 3.50 (1 H, p, *J* 6.5, 13-H), 3.42 (2 H, t, *J* 6.5, 9'-CH_2), 2.31 (1 H, dd, *J* 13.0, 7.5, 4-*H*H), 2.26 (1 H, dd, *J* 13.0, 7.5, 4-*H*H), 2.18 (3 H, s, 3-CH_3), 2.15 (1 H, m, 9-*H*H), 2.05 (1 H, m, 12-H), 2.00 (1 H, m, 9-H*H*), 1.63 (1 H, m, 8-H), 1.62 (6 H, m, CH_2 x 3), 1.50 (1 H, m, 6-*H*H), 1.49 (1 H, m, 6-H*H*), 1.29 (8 H, m, CH_2 x 4), 1.16 (3 H, d, *J* 6.5, 14-H_3), 0.98 (3 H, d, *J* 6.5, 12-CH_3), 0.90 (3 H, d, *J* 6.5, 8-CH_3); 166.7 (C-1), 159.2 (Ar-C), 155.8 (C-3), 134.2 (C-10), 130.9 (Ar-C), 130.7 (C-11), 129.4 (Ar-C), 118.5 (C-2), 113.9 (Ar-C), 76.6 (C-7), 72.6 (OCH_2Ar), 71.3 (C-13), 70.7 (C-5), 70.3 (C-9'), 64.1 (C-1'), 55.4 (OMe), 49.6 (C-4), 45.2 (C-12), 39.4 (C-8), 39.2 (C-6), 35.8, (C-9), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.8 (CH_2), 26.3 (CH_2), 26.1 (CH_2), 20.4 (C-14), 19.1 (3-CH_3), 16.9 (12-CH_3), 15.6 (8-CH_3); Found (MALDI): 599.3927 $[M+Na]^+$, ($C_{34}H_{56}NaO_7$ requires 599.3918).

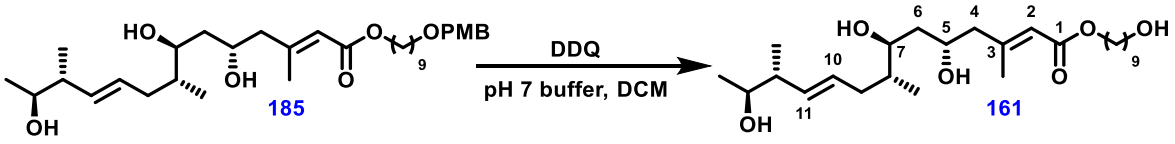
***para*-Methoxybenzyl ether of (*R*)-des-6-hydroxy-desepoxy mupirocin W4-OH 186**



186

ν_{max} (neat)/cm^{-1} 3327, 2950, 2929, 2850, 1769, 1642, 1513, 1462, 1245, 1145, 1093; $[\alpha]_D^{25}$= +6 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.25 (2 H, d, *J* 8.5, Ar-H), 6.87 (2 H, d, *J* 8.5, Ar-H), 5.73 (1 H, s, 2-H), 5.54 (1 H, m, 11-H), 5.36 (1 H, dd, *J* 15.0, 8.5, 10-H), 4.42 (2 H, s, OCH_2Ar), 4.15 (1 H, dp, *J* 12.0, 4.5, 5-H) 4.08 (2 H, t, *J* 6.5, 1'-CH_2), 3.80 (3 H, s, OMe), 3.76 (1 H, m, 7-H), 3.50 (1 H, p, *J* 6.5, 13-H), 3.43 (2 H, t, *J* 6.5, 9'-CH_2), 2.34 (1 H, dd, *J* 13.5, 8.5, 4-*H*H), 2.27 (1 H, dd, *J* 13.5, 5.0, 4-H*H*), 2.20 (3 H, s, 3-CH_3), 2.17 (1 H, m, 9-*H*H), 2.07 (1 H, m, 12-H), 2.03 (1 H, m, 9-H*H*), 1.63 (1 H, m, 8-H), 1.62 (6 H, m, CH_2 x 3), 1.50 (1 H, m, 6-*H*H), 1.49 (1 H, m, 6-H*H*), 1.37-1.26 (8 H, m, CH_2 x 4), 1.16 (3 H, d, *J* 6.5, 14-H_3), 0.98 (3 H, d, *J* 6.5, 12-CH_3), 0.88 (3 H, d, *J* 6.5, 8-CH_3);δ_C (125 MHz, $CDCl_3$) 166.7 (C-1), 159.2 (Ar-C), 156.1 (C-3), 134.1 (C-10), 130.9 (Ar-C), 130.6 (C-11), 129.4 (Ar-C), 118.4 (C-2), 113.9 (Ar-C), 72.6 (OCH_2Ar), 72.4 (C-7), 71.4 (C-13), 70.3 (C-9'), 66.9 (C-5), 64.1 (C-1'), 55.4 (OMe), 49.1 (C-4), 45.1 (C-12), 39.4 (C-6), 38.9 (C-8), 36.3, (C-9), 29.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.8 (CH_2), 26.3 (CH_2), 26.1 (CH_2), 20.6 (C-14), 19.2 (3-CH_3), 16.9 (12-CH_3), 15.7 (8-CH_3); Found (MALDI): 599.3927 $[M+Na]^+$, ($C_{34}H_{56}NaO_7$ requires 599.3918).

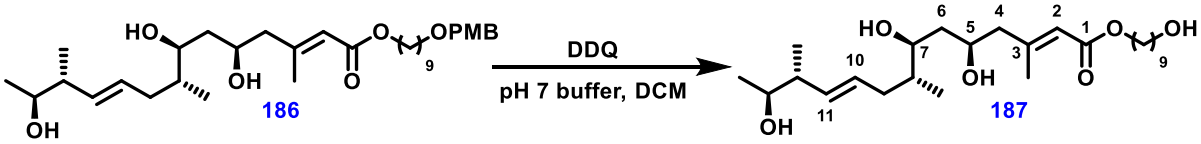
(*S*)-Des-6-hydroxy-desepoxy mupirocin W4-OH 161



Alcohol **185** (25 mg, 0.04 mmol) was dissolved in DCM (0.95 mL) and pH 7 buffer (0.05 mL). DDQ (10 mg, 0.042 mmol) was added and the reaction mixture was stirred in a sealed vial for 4 h, before the reaction was quenched by addition of water (*ca*. 0.5 mL). The layers were separated, and the aqueous layer extracted with Et_2O (3 x 1 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (100% EtOAc) to give **161** as a pale yellow oil (10.6 mg, 58%); ν_{max} (neat)/cm^{-1} 3355, 2950, 2929, 2850, 1713, 1550, 1420, 1273, 1151; $[\alpha]_D^{25}$= +30 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 5.75 (1 H, s, 2-H), 5.54 (1 H, m, 10-H), 5.34 (1 H, dd, *J* 15.5, 8.5, 11-H), 4.07 (2 H, m, 1'-H_2), 4.03 (1 H, m, 5-H), 3.72 (1 H, ddd, *J* 10.5, 5.5, 2.5, 7-H), 3.62 (2 H, t, *J* 6.5, 9'-H_2), 3.51 (1 H, p, *J* 6.5, 13-H), 2.32 (1 H, m, 4-*H*H), 2.30 (1 H, m, 4-H*H*), 2.19 (3 H, s, 3-CH_3), 2.14 (1 H, m, 9-*H*H), 2.07 (1 H, m, 12-H), 2.00 (1 H, m, 9-H*H*), 1.64 (1 H, m, 8-H), 1.64-1.48 (8 H, m, 4 x CH_2), 1.58 (1 H, m, 6-*H*H), 1.49 (1 H, m, 6-H*H*), 1.38-1.26 (10 H, m, 5 x CH_2), 1.16 (3 H, d, *J* 6.5, 14-H_3), 0.98 (3 H, d, *J* 6.5, 12-CH_3), 0.90 (3 H, d, *J* 6.5, 8-CH_3); δ_C (125

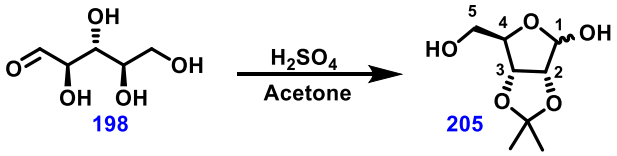
MHz, $CDCl_3$) 166.7 (C-1), 155.8 (C-3), 134.2 (C-10), 130.7 (C-11), 119.3 (C-2), 76.7 (C-7), 71.3 (C-13), 70.7 (C-5), 64.0 (C-1'), 63.1 (C-9'), 49.6 (C-4), 45.3 (C-12), 39.4 (C-8), 39.3 (C-6), 35.8 (CH_2), 32.9 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.8 (CH_2), 26.0 (CH_2), 25.8 (CH_2), 20.5 (C-14), 19.2 (3-CH_3), 16.9 (12-CH_3), 15.6 (8-CH_3); Found (ESI): 479.3339 $[M+Na]^+$, ($C_{26}H_{48}NaO_6$ requires 479.3343).

(*R*)-Des-6-hydroxy-desepoxy mupirocin W4-OH 187



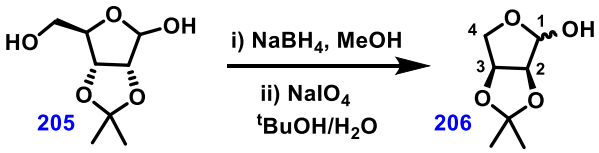
Alcohol **186** (35 mg, 0.06 mmol) was dissolved in DCM (0.95 mL) and pH 7 buffer (0.05 mL). DDQ (10 mg, 0.042 mmol) was added and the reaction mixture was stirred in a sealed vial for 4 h, before the reaction was quenched by addition of water (*ca*. 0.5 mL). The layers were separated, and the aqueous layer extracted with Et_2O (3 x 1 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (100% EtOAc) to give **187** as a pale yellow oil (17.2 mg, 63%); ν_{max} (neat)/cm^{-1} 3355, 2950, 2929, 2850, 1713, 1550, 1420, 1273, 1151; $[\alpha]_D^{25}$= +8 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 5.74 (1 H, s, 2-H), 5.55 (1 H, dt, *J* 15.0, 7.0, 10-H), 5.34 (1 H, dd, *J* 15.0, 8.5, 11-H), 4.16 (1 H, m, 5-H), 4.08 (2 H, m, 1'-H_2), 3.77 (1 H, ddd, *J* 9.0, 6.5, 2.5, 7-H), 3.63 (2 H, t, *J* 6.5, 9'-H_2), 3.51 (1 H, p, *J* 6.5, 13-H), 2.34 (1 H, m, 4-*H*H), 2.28 (1 H, dd, *J* 13.5, 4.5, 4-H*H*), 2.19 (3 H, s, 3-CH_3), 2.18 (1 H, m, 9-*H*H), 2.06 (1 H, m, 12-H), 2.04 (1 H, m, 9-H*H*), 1.69 (1 H, m, 8-H), 1.66-1.53 (8 H, m, 4 x CH_2), 1.58 (1 H, m, 6-*H*H), 1.49 (1 H, m, 6-H*H*), 1.38-1.26 (8 H, m, 4 x CH_2), 1.16 (3 H, d, *J* 6.5, 14-H_3), 0.98 (3 H, d, *J* 6.5, 12-CH_3), 0.89 (3 H, d, *J* 6.5, 8-CH_3); δ_C (125 MHz, $CDCl_3$) 166.7 (C-1), 156.2 (C-3), 134.1 (C-10), 130.6 (C-11), 118.4 (C-2), 72.4 (C-7), 71.5 (C-13), 66.9 (C-5), 64.0 (C-1'), 63.1 (C-9'), 49.1 (C-4), 45.2 (C-12), 39.4 (C-8), 38.9 (C-6), 36.3 (CH_2), 32.9 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 26.0 (CH_2), 25.8 (CH_2), 20.6 (C-14), 19.2 (3-CH_3), 16.9 (12-CH_3), 15.7 (8-CH_3); Found (ESI): 479.3340 $[M+Na]^+$, ($C_{26}H_{48}NaO_6$ requires 479.3343).

(2*S*,3*S*)-Acetonide of (3*R*,4*S*,5*R*)-5-(hydroxymethyl)tetrahydrofuran-2,3,4-triol 205



To a stirred suspension of D-ribose **198** (10.0 g, 66.6 mmol) in acetone (100 mL) was added conc. H_2SO_4 (0.2 mL) and the resulting solution stirred at RT for 2 h. After this time the solution was neutralised with solid $NaHCO_3$ (*ca.* 20 g), filtered and the solvent removed *in vacuo* to give a viscous oil. This was purified by column chromatography (80% EtOAc in petrol) to give **205** as a viscous colourless oil (12.2 g, 97%); δ_H (400 MHz, $CDCl_3$) 5.42 (1 H, d, *J* 6.0, 1-H), 4.84 (1 H, dd, *J* 6.0, 1.0, 4-H), 4.58 (1 H, d, *J* 6.0, 2-H), 4.41 (1 H, t, *J* 3.0, 3-H), 3.80-3.66 (2 H, m, 5-H_2), 1.49 (3H, s, $C(CH_3)_2$), 1.32 (3 H, s, $C(CH_3)_2$); δ_C (101 MHz, $CDCl_3$) 112.3 (C-1) , 103.2 ($\underline{C}(CH_3)_2$), 88.0 (C-3), 87.0 (C-2), 81.8 (C-4), 63.8 (C-5), 26.5 (CH_3), 24.9 (CH_3). Data in accordance with the literature.[216] Data reported for the major compound.

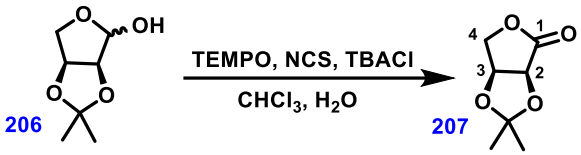
(2*S*,3*S*)-Acetonide of (3*S*,4*S*)-tetrahydrofuran-2,3,4-triol 206



Lactol **205** (12.7 g, 66.7 mmol) was dissolved in MeOH (75 mL) and cooled to 0 °C. $NaBH_4$ (3.79 g, 100.1 mmol) was added portion wise over 10 mins. The cold bath was removed and the reaction stirred at RT for 1 h, after which time the solvent was removed *in vacuo* to give a white foam. To this was added H_2O (60 mL) and ^{t}BuOH (90 mL) and the foam dissolved by sonication. To this stirred solution was added $NaIO_4$ (50.0 g, 240 mmol) portionwise over 10 mins and the reaction mixture stirred at RT for 4 h, before dilution with DCM (100 mL) and neutralisation by solid $NaHCO_3$ (*ca*. 40 g). This was filtered and the filtrate extracted with DCM (3 x 50 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give a colourless oil. This was purified by column chromatography (50% EtOAc in petrol) to give **206** as a colourless oil (10.2 g, 44%); δ_H (400 MHz, $CDCl_3$) 5.41 (1 H, d, *J* 2.0, 1-H), 4.83 (1 H, dd, *J* 6.0, 3.5, 3-H), 4.57 (1 H, d, *J* 6.0, 2-H), 4.07 (1 H, dd, *J* 10.5, 3.5, 4-*H*H), 4.01 (1 H, d, *J* 10.5, 4-H*H*), 1.46 (3 H, s, $C(CH_3)_2$), 1.31 (3 H, s, $C(CH_3)_2$); δ_C (101 MHz, $CDCl_3$) 112.4 ($\underline{C}(CH_3)_2$), 102.0 (C-1),

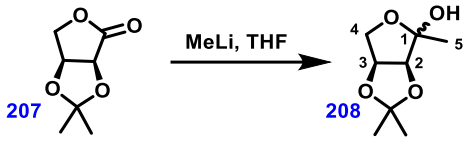
85.3 (C-2), 80.1 (C-3), 72.1 (C-4), 26.3 (CH_3), 24.9 (CH_3). Data in accordance with the literature.[217] Data reported for the major compound.

(2*S*,3*S*)-Acetonide of L-erythrono-1,4-lactone 207



Lactol **206** (10.2 g, 63.7 mmol) was dissolved in $CHCl_3$ (250 mL) and a solution of TEMPO (0.90 g, 5.73 mmol), $NaHCO_3$ (8.02 g, 95.5 mmol), K_2CO_3 (1.32 g, 9.56 mmol), TBACl (1.59 g, 5.73 mmol) in H_2O (150 mL) was added followed by NCS (15.3 g, 114.6 mmol). The reaction was stirred overnight at RT before the addition of sat. aq. $Na_2S_2O_3$ (100 mL). The layers were separated, and the aqueous layer extracted with EtOAc (3 x 100 mL), combined and dried ($MgSO_4$) and the solvent removed *in vacuo* to give an orange oil. This was purified by column chromatography (30% EtOAc in petrol) to give **207** as a white crystalline solid (4.66 g, 74%). M.p. 62-64 °C (lit. 60-62 °C); $[\alpha]_D^{23}$ +124.0 (*c* 1.0, $CHCl_3$), lit. $[\alpha]_D^{20}$ +118.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.88 (1H, ddd, *J* 5.5, 4.0, 0.5, 2-H), 4.74 (1H, d, *J* 5.5, 3-H), 4.47 (1H, d, *J* 11.0, 4-*H*H), 4.40 (1H, dd, *J* 11.0, 4.0, 4-H*H*), 1.49 (3H, s, $C(CH_3)_2$), 1.40 (3H, s, $C(CH_3)_2$); δ_C (101 MHz, $CDCl_3$) 174.1 (C-1), 114.2 ($\underline{C}(CH_3)_2$), 75.6 (C-2), 74.8 (C-3), 70.3 (C-4), 26.9 (CH_3), 25.8 (CH_3). Data in accordance with the literature.[218]

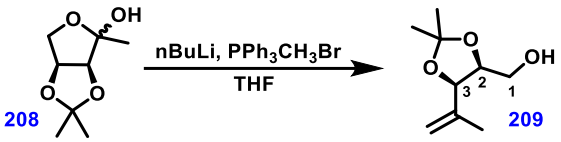
(2*S*,3*S*)-Acetonide of 1-methyltetrahydrofuran-1-ol 208



To a stirred solution of lactol **207** (9.32 g, 58.9 mmol) in anhydrous THF (170 mL) was added MeLi (40.5 mL, 64.8 mmol) dropwise at -78 °C and the reaction mixture stirred for 3 h at this temperature. After this time aq. NH_4Cl (100 mL) was added and the mixture extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give **208** as an orange oil (9.33 g, 91%); δ_H (400 MHz, $CDCl_3$) 4.86 (1H, dd, *J* 6.0, 4.0, 3-H), 4.41 (1H, d, *J* 6.0, 2-H), 4.01 (1H, dd, *J* 10.5, 4.0, 4-*H*H), 3.92 (1H, d, *J* 10.5, 4-H*H*), 2.09 (1H, s, OH), 1.54 (3H, s, 5-CH_3), 1.48 (3H, s, $C(CH_3)_2$), 1.33 (3H, s, $C(CH_3)_2$); δ_C (101

MHz, $CDCl_3$) 112.6 (<u>C</u>$(CH_3)_2$), 106.4 (C-1), 85.1 (C-2), 81.0 (C-3), 71.2 (C-4), 26.5 (C(<u>C</u>$H_3)_2$), 25.1 (C(<u>C</u>$H_3)_2$), 22.6 (C-5); Found (ESI): 198.0825 $[M+Na]^+$,($C_8H_{14}O_4Na$ requires 198.0818). Data in accordance with the literature.[219]

(2*S*,3*R*)-Acetonide of 4-methylpent-4-en-1-ol 209



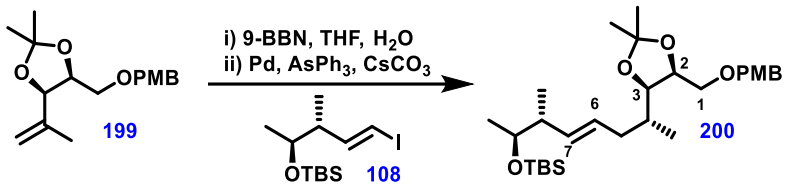
To a suspension of methyltriphenylphosphonium bromide (18.5 g, 51.7 mmol) in anhydrous THF (150 mL) at −78 °C was added dropwise *n*BuLi (2.5 M in hexanes, 20.7 mL, 51.7 mmol). The reaction was stirred at 0 °C for 30 mins, then cooled to −78 °C. Lactol **208** (3.00 g, 17.2 mmol) in anhydrous THF (10 mL) was added dropwise, and the reaction was stirred for 1 h, then heated to reflux for 24 h. The reaction was cooled to RT and quenched with water (50 mL) and the phases were separated. The aqueous layer was extracted with EtOAc (3 × 50 mL), the combined organic extracts washed with brine (30 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give an orange oil which was purified by column chromatography (20-30% EtOAc in petrol) to give **209** as a colourless oil (2.60 g, 29%); $[\alpha]_D^{23}$ −91.0 (*c* 1.0, $CHCl_3$), lit. $[\alpha]_D^{20}$ −86.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.13 (1 H, m, 5-*H*H), 4.96 (1 H, m, 5-H*H*), 4.61 (1 H, d, *J* 6.5, 3-H), 4.28 (1 H, ddd, *J* 8.0, 6.5, 4.5, 2-H), 3.55 – 3.41 (2 H, m, 1-H_2), 1.87 (1 H, s, OH), 1.74 (3 H, m, 6-H_3), 1.52 (3 H, q, *J* 1.0, $C(CH_3)_2$), 1.40 (3 H, q, *J* 1.0, $C(CH_3)_2$); δ_C (101 MHz, $CDCl_3$) 139.7 (C-4), 112.3 (C-5), 108.8 (<u>C</u>$(CH_3)_2$), 79.3 (C-3), 77.8 (C-2), 62.3 (C-1), 27.9 (C(<u>C</u>$H_3)_2$), 25.6 (C(<u>C</u>$H_3)_2$), 20.3 (C-6). Data are consistent with literature.[88]

(2*S*,3*R*)-Acetonide of 1-(4-methoxybenzyloxy)-4-methylpent-4-ene 199

NaH (60% in mineral oil, 208 mg, 5.21 mmol) was suspended in anhydrous THF (5.5 mL) and DMSO (7.5 mL) under N_2 at 0 °C. To this was added alcohol **209** (750 mg, 4.74 mmol) in anhydrous THF (2 mL). The reaction was stirred at this temperature for 1 h, then PMBCl (0.71

mL, 5.21 mmol) and TBAI (174 mg, 0.47 mmol) were added. The reaction was allowed to slowly warm to RT over 16 h, after which time H_2O (10 mL) was added. The reaction mixture was extracted with DCM (3 x 15 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to give a yellow oil. This was purified by column chromatography (20% EtOAc in petrol) to give **199** as a colourless oil (860 mg, 62%); ν_{max} (neat)/cm^{-1} 2954, 2873, 1616, 1513, 1244; $[\alpha]_D^{23}$ −16.9 (*c* 0.65, $CHCl_3$), lit. $[\alpha]_D^{20}$ −19.8 (*c* 0.65, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.24 (2 H, d, *J* 9.0, 9-H_2), 6.86 (2 H, d, *J* 9.0, 10-H_2), 5.07 (1 H, m, 5-*H*H), 4.96 – 4.89 (1 H, m, 5-H*H*), 4.59 (1 H, d, *J* 6.5, 3-H), 4.46 (1 H, d, *J* 11.5, 7-*H*H), 4.40 (1 H, d, *J* 11.5, 7-H*H*), 4.39 (1 H, td, *J* 6.5, 5.5, 2-H), 3.80 (3 H, s, OMe), 3.37 (1 H, d, 1.5, 1-*H*H), 3.36 (1 H, d, 0.5, 1-H*H*), 1.75 – 1.69 (3 H, m, 6-H_3), 1.49 (3 H, d, *J* 0.5, $C(CH_3)_2$), 1.38 (3 H, d, *J* 0.5, $C(CH_3)_2$);); δ_C (101 MHz, $CDCl_3$) 159.3 (C-11), 140.6 (C-4), 130.3 (C-8), 129.5 (C-9), 113.9 (C-5), 112.3 (C-10), 108.6 ($\underline{C}(CH_3)_2$), 79.9 (C-3), 76.8 (C-2), 73.1 (C-9), 69.5 (C-1), 55.4 (OMe), 27.7 ($C(\underline{C}H_3)_2$), 25.4 ($C(\underline{C}H_3)_2$), 20.3 (C-6). Found (ESI): 315.1571 $[M+Na]^+$, ($C_{17}H_{24}O_4Na$ requires 315.1567).

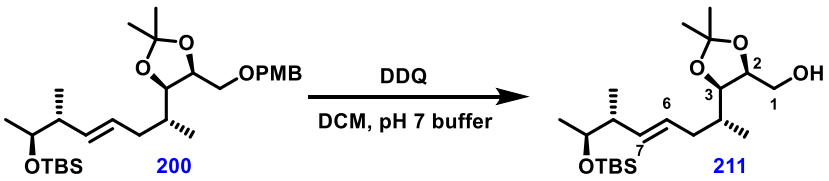
(8*R*,9*S*)-Acetonide of (2*S*,3*R*,7*R*,4*E*)-2-(*tert*-butyldimethylsilyloxy)-3,7-dimethyl-10-(4-methoxybenzyloxy)dec-4-ene 200



To a stirred solution of alkene **199** (616 mg, 2.11 mmol) in degassed THF (8 mL) at -78 °C was added 9-BBN (12.68 mL, 6.24 mmol) under N_2. The reaction was stirred at RT for 16 h before the addition of degassed H_2O (8 mL) and stirred for 1 h. In a separate flask vinyl iodide **108** (600 mg, 1.76 mmol) was dissolved in degassed DMF (15 mL) and Cs_2CO_3 (2.07 g, 6.35 mmol), $Pd(dppf)Cl_2$ (322 mg, 0.44 mmol) and $AsPh_3$ (135 mg, 0.44 mmol) were added and the reaction mixture stirred for 10 mins. The borane solution was then added to this mixture and the reaction was stirred at RT for 8 h before the addition of H_2O (15 mL). The reaction mixture was filtered through Celite and washed with H_2O (5 x 15 mL). The organic extract was dried ($MgSO_4$) and the solvent removed *in vacuo* to give a brown oil. This was purified by column chromatography (10% EtOAc in petrol) to give **200** as a colourless oil (449 mg, 42%); $[\alpha]_D^{23}$ −12.0(*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.25 (1 H, d, *J* 8.5, Ar-H), 6.89 (1 H, d, *J* 8.5, Ar-H), 5.51

– 5.30 (2 H, m, 7-H + 6-H), 4.49 (2 H, s, 13-H_2), 4.20 (1 H, dt, *J* 7.0, 5.0, 2-H), 3.80 (4 H, m, OCH_3, 3-H), 3.70 (1 H, qd, *J* 6.0, 4.0, 9-H), 3.51 (1 H, dd, *J* 10.0, 5.0, 1-*H*H), 3.39 (1 H, dd, *J* 10.0, 7.0, 1-H*H*), 2.34 (1 H, m, 5-*H*H), 2.18 (1 H, m, 8-H), 1.91 (1 H, m, 5-H*H*), 1.66 (1 H, m, 4-H), 1.40 (3H, s, $C(CH_3)_2$), 1.32 (3H, s, $C(CH_3)_2$), 1.04 (3H, d, *J* 6.5, 10-H_3), 0.97 (3H, d, *J* 7.0, 11-CH_3), 0.88 (9H, s, $SiC(CH_3)_3$), 0.86 (3H, dd, *J* 12.5, 6.5, 12-CH_3), 0.02 (6H, s, $Si(CH_3)_3$); δ_C (101 MHz, $CDCl_3$) 159.2 (Ar-C), 135.1 (C-7), 130.3 (Ar-C), 129.5 (Ar-C), 127.1 (C-6), 113.9 (Ar-C), 107.9 ($\underline{C}(CH_3)_2$), 81.4 (C-3), 76.6 (C-2), 73.2 (C-13), 72.1 (C-9), 69.2 (C-1), 55.4 (OMe), 44.4 (C-8), 37.1 (C-5), 32.3 (C-4), 28.5 ($C(\underline{C}H_3)_2$), 26.0 ($C(\underline{C}H_3)_2$), 26.0 ($SiC(\underline{C}H_3)_3$), 20.5 (C-10), 18.3 ($Si\underline{C}(CH_3)_3$), 16.1 (C-11), 16.0 (C-12), -4.2 ($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$); ν_{max} (neat)/cm^{-1} 2960, 2929, 2856, 1632, 1513, 1369, 1247, 1090; Found (ESI): 530.3359 $[M+Na]^+$,($C_{29}H_{50}O_5SiNa$ requires 530.3353).

(8*R*,9*S*)-Acetonide of (2*S*,3*R*,7*R*,4*E*)-2-(*tert*-butyldimethylsilyloxy)-3,7-dimethyldec-4-ene 211



PMB ether **200** (100 mg, 0.20 mmol) was dissolved in DCM (1.5 mL) and pH 7 buffer (0.1 mL) added. To this mixture was added DDQ (45 mg, 0.40 mmol) and the reaction stirred at RT for 3 h before the addition of aq. $NaHCO_3$ (5 mL). The reaction mixture was extracted with DCM (3 x 5 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent *removed in vacuo* to give an orange oil. This was purified by column chromatography (10% EtOAc in petrol) to give **211** as a colourless oil (34 mg, 44%); $[\alpha]_D^{23}$ −26.0 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5.52-5.32 (2 H, m, 7-H + 6-H), 4.13 (1 H, q, *J* 6.0, 9-H), 3.84 (1 H, ddd, *J* 10.5, 5.5, 2.0, 3-H), 3.69 (1 H, qd, *J* 6.0, 4.0, 9-H), 3.64-3.58 (2 H, m, 10-H_2), 2.38 (1 H, m, 5-*H*H), 2.14 (1 H, m, 8-H), 1.98-1.87 (2 H, m, 5-H*H*, OH), 1.70 (1 H, m, 4-H), 1.47 (3 H, s, $C(CH_3)_2$), 1.36 (3 H, s, $C(CH_3)_2$), 1.03 (3 H, d, *J* 6.0, 10-H_3), 0.96 (3 H, d, *J* 7.0, 11-H_3), 0.88 (9 H, s, $SiC(CH_3)_3$), 0.87 (3 H, dd, *J* 6.5, 12-H_3), 0.03 (6 H, s, $Si(CH_3)_2$); δ_C (101 MHz, $CDCl_3$) 135.4 (C-7), 126.7 (C-6), 108.3 ($\underline{C}(CH_3)_2$), 81.1 (C-3), 77.9 (C-2), 72.1 (C-9), 61.7 (C-1), 44.4 (C-8), 37.1 (C-5), 32.0 (C-4), 28.7 ($C(\underline{C}H_3)_2$), 26.0 ($C(\underline{C}H_3)_2$), 26.0 ($SiC(\underline{C}H_3)_3$), 20.8 (C-10), 18.3 ($Si\underline{C}(CH_3)_3$), 15.8 (C-11), 15.8 (C-

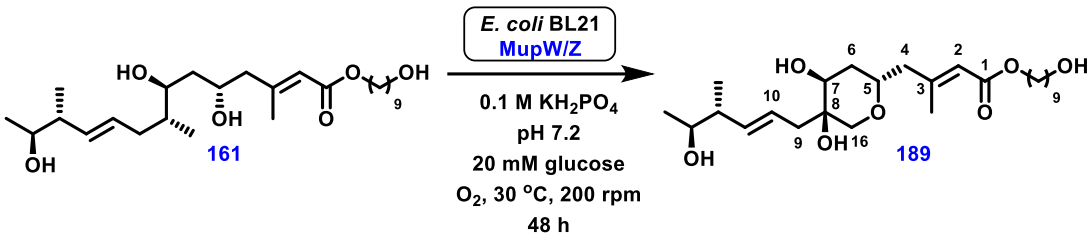
12), -4.2 ($Si(CH_3)_2$), -4.7 ($Si(CH_3)_2$); ν_{max} (neat)/cm^{-1} 3443 (br), 2955, 2929, 2856, 1461, 1370 , 1250, 1063, 1032; Found (ESI): 410.2784 $[M+Na]^+$,($C_{21}H_{42}O_4SiNa$ requires 410.2778).

General Procedure for Biotransformations

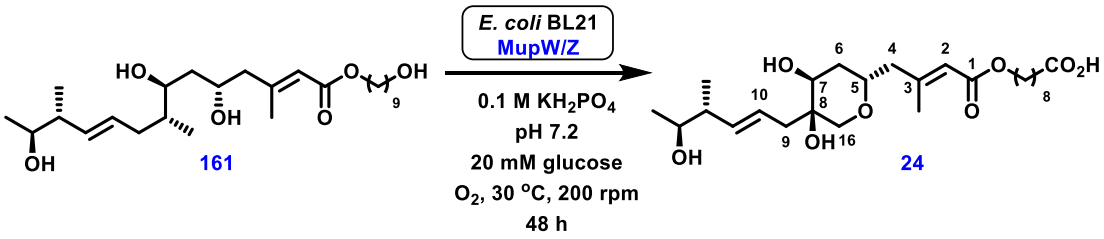
Agar was prepared and the plates streaked with *E.coli* BL21 containing the plasmid for *mupW* and *mupZ* expression. This plate was incubated overnight at 37 °C. Auto-induction media (200 mL) was prepared and autoclaved for 3 h. A single colony was selected and added to the auto-induction media, to which were added carbenicillin (100 µl) and kanamycin (100 µl) to eliminate any bacteria which did not contain the desired plasmid. The cultures were then incubated for 16 h at 37 °C and shaken at 200 rpm. The culture was separated into three equal portions, one as a negative control (enzymes boiled at 95 °C for 10 mins and with substrate) and to two were added substrate. In addition, pH 7.3 buffer (2 mL) and glucose (50 µl) were added. The reaction mixtures were shaken at 200 rpm for 16 h at 30 °C. After this time an aliquot of the reaction mixture (1 mL) was centrifuged and the supernatant was suspended in MeCN. The mixture was vortexed and the organic layer concentrated under a stream of N_2. The resulting product was analysed by HP-LC.

General Procedure for Bacterial Growth

LB agar was inoculated with Tetracycline (5 µg/mL) and the plate streaked with *Pseudomonas fluorescens* ΔmupA-pJH_2. The plate was incubated overnight at 37 °C and a single colony was selected. This was added to a seed culture which had been made from 1% Tryptone, 0.5% yeast extract, and 0.5% NaCl. This was shaken overnight at 200 rpm and 30 °C. Fermentation culture (2.13 L) was prepared by the addition of Tryptone (25 g), Yeast extract (12.5 g) and NaCl (12.5 g) in water (2.13 L). Into 24 flasks was added 85 mL of this mixture and autoclaved overnight. A solution of glucose was made by dissolving glucose (100 g) in water (100 mL) and making up to 250 mL. This was autoclaved overnight. To the fermentation culture was added the seed culture (5 mL), glucose solution (10 mL) and IPTG (5 µL). This mixture was shaken at 200 rpm and 22 °C for 50 h. After this time the combined fermentation mixtures were centrifuged and the supernatant extract with EtOAc.

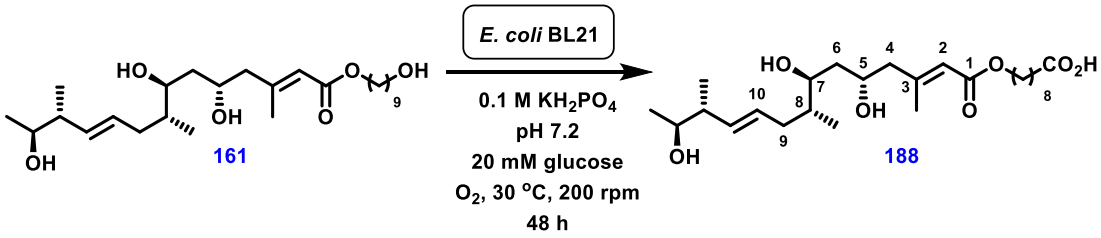


δ_H (700 MHz, CD_3OD) 5.74 (1 H, s, 2-H), 5.58 (1 H, dt, *J* 15.0, 7.5, 10-H), 5.50 (1 H, dd, *J* 15.0, 7.5, 11-H), 4.09 (2 H, t, *J* 6.5, OCH_2), 3.96 (1 H, dddd, *J* 11.0, 8.0, 5.0, 3.0, 5-H), 3.76 (1 H, t, *J* 3.5, 7-H), 3.64 (1 H, p, *J* 6.0, 13-H), 3.56 (2 H, t, *J* 6.5, OCH_2), 3.54 (1 H, d, *J* 11.5, 16-*H*H), 3.41 (1 H, d, *J* 11.5, 16-H*H*), 2.38 (2 H, m, 9-H_2), 2.36 (1 H, m, 4-*H*H), 2.27 (1 H, dd, *J* 14.0, 5.0, 4-H*H*), 2.20 (1 H, m, 12-H), 2.18 (3 H, s, 3-CH_3), 1.72 (1 H, dt, *J* 14.0, 3.5, 6-*H*H), 1.67 (1 H, m, 6-H*H*), 1.67 (2 H, m, CH_2), 1.55 (2 H, m, CH_2), 1.36 (10 H, m, 5 x CH_2), 1.13 (3 H, d, *J* 6.5, 14-H_3), 1.02 (3 H, d, *J* 6.5, 12-CH_3); δ_C (175 MHz, CD_3OD) 168.3 (C-1), 158.2 (C-3), 137.4 (C-11), 126.1 (C-10), 118.5 (C-2), 72.1 (C-13), 71.2 (C-5), 71.1 (C-7), 70.4 (C-16), 70.0 (C-8), 64.9 (OCH_2), 63.0 (OCH_2), 47.2 (C-4), 45.5 (C-12), 39.8 (C-9), 37.0 (C-6), 33.7 (CH_2), 30.6 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 20.3 (C-14), 19.2 (3-CH_3), 16.5 (12-CH_3); Found (ESI): 471.32 $[M+H]^+$,($C_{26}H_{47}O_7$ requires 471.3244).

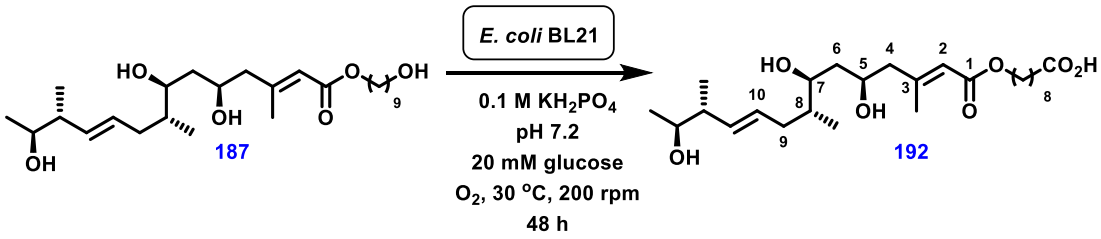


δ_H (700 MHz, CD_3OD) 5.74 (1 H, s, 2-H), 5.58 (1 H, dt, *J* 15.0, 7.5, 10-H), 5.50 (1 H, dd, *J* 15.0, 7.5, 11-H), 4.09 (2 H, t, *J* 6.5, OCH_2), 3.96 (1 H, dddd, *J* 11.0, 8.0, 5.0, 3.0, 5-H), 3.75 (1 H, t, *J* 3.5, 7-H), 3.63 (1 H, p, *J* 6.0, 13-H), 3.54 (1 H, d, *J* 11.5, 16-*H*H), 3.41 (1 H, d, *J* 11.5, 16-H*H*), 2.38 (2 H, m, 9-H_2), 2.36 (1 H, m, 4-*H*H), 2.27 (1 H, dd, *J* 14.0, 5.0, 4-H*H*), 2.22 (2 H, m, CH_2CO_2H), 2.19 (1 H, m, 12-H), 2.18 (3 H, s, 3-CH_3), 1.71 (1 H, dt, *J* 14.0, 3.5, 6-*H*H), 1.67 (1 H, m, 6-H*H*), 1.65 (2 H, m, CH_2), 1.61 (2 H, m, CH_2), 1.36 (8 H, m, 4 x CH_2), 1.12 (3 H, d, *J* 6.5, 14-H_3), 1.02 (3 H, d, *J* 6.5, 12-CH_3); δ_C (175 MHz, CD_3OD) 180.1 (CO_2H), 165.5 (C-1), 158.1 (C-3), 137.4 (C-11), 126.1 (C-10), 118.5 (C-2), 72.1 (C-13), 71.2 (C-5), 71.1 (C-7), 70.4 (C-16), 70.1 (C-8), 64.9 (OCH_2), 47.2 (C-4), 45.0 (C-12), 39.8 (C-9), 37.5 (CH_2COOH), 36.9 (C-6), 33.7 (OCH_2),

30.5 (CH_2), 30.4 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 27.1 (CH_2), 27.1 (CH_2), 20.2 (C-14), 19.2 (3-CH_3), 16.5 (12-CH_3); Found (ESI): 485.30 $[M+H]^+$,($C_{26}H_{45}O_8$ requires 485.3036).

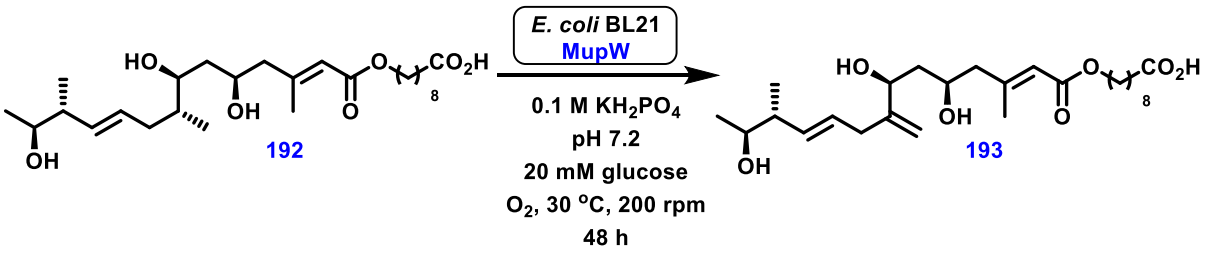


δ_H (700 MHz, CD_3OD) 5.76 (1 H, s, 2-H), 5.45 (1 H, dt, *J* 15.5, 7.0, 10-H), 5.40 (1 H, dd, *J* 15.5, 7.5, 11-H), 4.09 (2 H, t, *J* 6.5, OCH_2), 4.04 (1 H, tt, *J* 8.5, 5.0, 5-H), 3.63 (2 H, m, 13-H + 7-H), 2.39 (1 H, dd, *J* 13.5, 4.5, 4-*H*H), 2.26 (2 H, m, CH_2CO_2H), 2.24 (1 H, m, 4-H*H*), 2.20 (3 H, s, 3-CH_3), 2.18 (2 H, m, 12-H + 9-*H*H), 1.89 (1 H, dt, *J* 14.5, 7.5, 9-H*H*), 1.65 (2 H, m, CH_2), 1.63 (1 H, m, 6-*H*H), 1.60 (2 H, m, CH_2), 1.57 (1 H, m, 8-H), 1.55 (1 H, m, 6-H*H*), 1.35 (8 H, m, 4 x CH_2), 1.30 (2 H, m, CH_2), 1.11 (3 H, d, *J* 6.5, 14-H_3), 1.00 (3 H, d, *J* 6.5, 12-CH_3), 0.91 (3 H, d, *J* 6.5, 8-CH_3); δ_C (175 MHz, CD_3OD) 179.4 (CO_2H), 168.3 (C-1), 158.5 (C-3), 135.0 (C-10), 130.5 (C-11), 118.8 (C-2), 74.9 (C-7), 72.2 (C-13), 69.9 (C-5), 64.8 (OCH_2), 49.6 (C-4), 45.3 (C-12), 40.7 (C-6), 40.6 (C-8), 36.6 (CH_2CO_2H), 36.6 (C-9), 30.4 (CH_2), 30.3 (CH_2), 29.8 (CH_2), 27.1 (CH_2), 27.1 (CH_2), 26.7 (CH_2), 20.2 (C-14), 19.3 (3-CH_3), 16.6 (12-CH_3), 15.5 (8-CH_3); Found (ESI): 471.32 $[M+H]^+$,($C_{26}H_{47}O_7$ requires 471.3277).

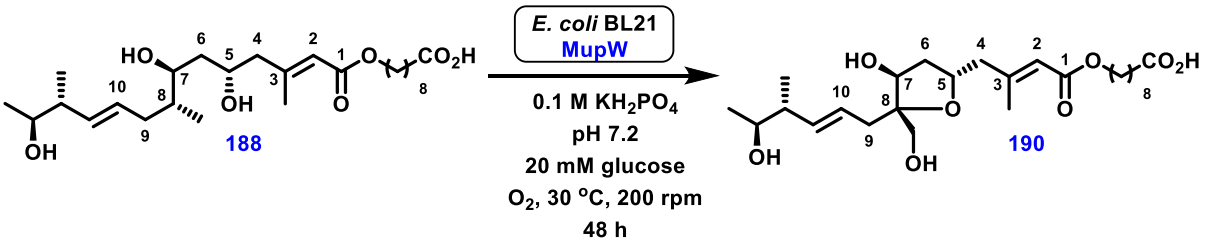


δ_H (700 MHz, CD_3OD) 5.75 (1 H, s, 2-H), 5.47 (1 H, dt, *J* 15.5, 6.5, 10-H), 5.43 (1 H, dd, *J* 15.5, 7.5, 11-H), 4.09 (3 H, t, *J* 6.5, OCH_2 + 5-H), 3.71 (1 H, ddd, *J* 10.0, 5.5, 2.0, 7-H), 3.63 (1 H, p, *J* 6.0, 13-H), 2.32 (2 H, m, 4-H_2), 2.25 (3 H, m, CH_2CO_2H + 9-*H*H), 2.21 (3 H, s, 3-CH_3), 2.16 (1 H, td, *J* 7.0, 5.0, 12-H), 1.89 (1 H, dt, *J* 14.0, 8.0, 9-H*H*), 1.66 (2 H, m, CH_2), 1.62 (1 H, ddd, *J* 14.5, 9.5, 2.0, 6-*H*H), 1.60 (2 H, m, CH_2), 1.57 (1 H, m, 8-H), 1.47 (1 H, ddd, *J* 14.0, 10.0, 2.5, 6-H*H*), 1.35 (8 H, m, 4 x CH_2), 1.30 (2 H, m, CH_2), 1.11 (3 H, d, *J* 6.5, 14-H_3), 1.00 (3 H, d, *J* 6.5, 12-CH_3), 0.91 (3 H, d, *J* 6.5, 8-CH_3); δ_C (175 MHz, CD_3OD) 179.7 (CO_2H), 168.4 (C-1), 158.7 (C-3), 134.9 (C-10), 130.6 (C-11), 118.6 (C-2), 72.5 (C-7), 72.2 (C-13), 67.4 (C-5), 64.8 (OCH_2), 50.6

(C-4), 45.3 (C-12), 41.8 (C-6), 41.0 (C-8), 36.9 (CH_2CO_2H), 36.9 (C-9), 30.4 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 29.8 (CH_2), 27.1 (CH_2), 26.6 (CH_2), 20.2 (C-14), 19.2 (3-CH_3), 16.6 (12-CH_3), 15.6 (8-CH_3); Found (ESI): 471.32 $[M+H]^+$,($C_{26}H_{47}O_7$ requires 471.3277).



δ_H (700 MHz, CD_3OD) 5.73 (1 H, s, 2-H), 5.47 (1 H, m, 10-H), 5.46 (1 H, m, 11-H), 5.08 (1 H, s, 8-*H*H), 4.84 (1 H, s, 8-H*H*), 4.31 (1 H, dd, *J* 9.5, 3.0, 7-H), 4.07 (2 H, t, *J* 6.5, OCH_2), 4.05 (1 H, m, 5-H), 3.62 (1 H, m, 13-H), 2.81 (1 H, dd, *J* 16.0, 5.5, 9-*H*H), 2.73 (1 H, dd, *J* 16.0, 5.5, 9-H*H*), 2.35 (1 H, m, 4-*H*H), 2.30 (1 H, m, 4-H*H*), 2.18 (3 H, s, 3-CH_3), 2.16 (1 H, m, 12-H), 2.16 (2 H, t, *J* 6.5, CH_2COOH), 2.06 (2 H, m, CH_2), 2.02 (2 H, m, CH_2), 1.61 (1 H, m, 6-HH), 1.60 (2 H, m, CH_2), 1.56 (1 H, m, 6-HH), 1.60 (2 H, m, CH_2), 1.32 (4 H, m, 2 x CH_2), 1.10 (3 H, d, *J* 6.5, 14-CH_3), 1.01 (3 H, d, *J* 6.5, 12-CH_3); Found (ESI): 469.31 $[M+H]^+$,($C_{26}H_{47}O_7$ requires 469.3121).



δ_H (700 MHz, CD_3OD) 5.75 (1 H, s, 2-H), 5.49 (2 H, m, 10-H + 11-H), 4.40 (1 H, dt, *J* 14.5, 6.0, 5-H), 4.21 (1 H, dd, *J* 6.0, 3.0, 5-H), 4.07 (2 H, t, *J* 6.5, OCH_2), 3.65 (1 H, m, 15-*H*H), 3.63 (1 H, m, 15-H*H*), 3.61 (1 H, m, 13-H), 2.41 (1 H, dd, *J* 13.5, 7.0, 4-*H*H), 2.35 (1 H, dd, *J* 13.5, 5.5, 4-H*H*), 2.25 (2 H, m, 9-H_2), 2.24 (2 H, m, CH_2COOH), 2.19 (3 H, s, 3-CH_3), 2.17 (1 H, m, 12-H), 1.97 (1 H, ddd, *J* 13.0, 5.5, 3.0, 6-*H*H), 1.87 (1 H, m, 6-H*H*), 1.66 (2 H, m, CH_2), 1.60 (2 H, m, CH_2), 1.37 (2 H, m, CH_2), 1.32 (6 H, m, 3 x CH_2), 1.11 (3 H, d, *J* 6.5, 14-CH_3), 1.01 (3 H, d, *J* 6.5, 12-CH_3); Found (ESI): 485.30 $[M+H]^+$,($C_{26}H_{45}O_8$ requires 485.3070).

CHAPTER 5: References

5. References

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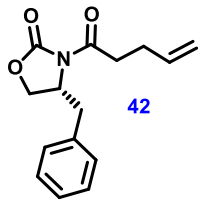
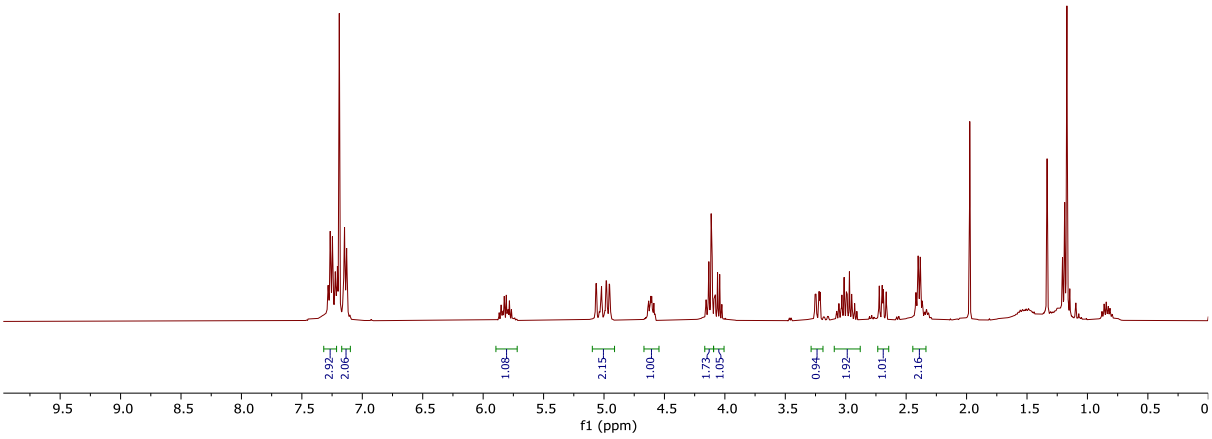
CHAPTER 6: Appendix

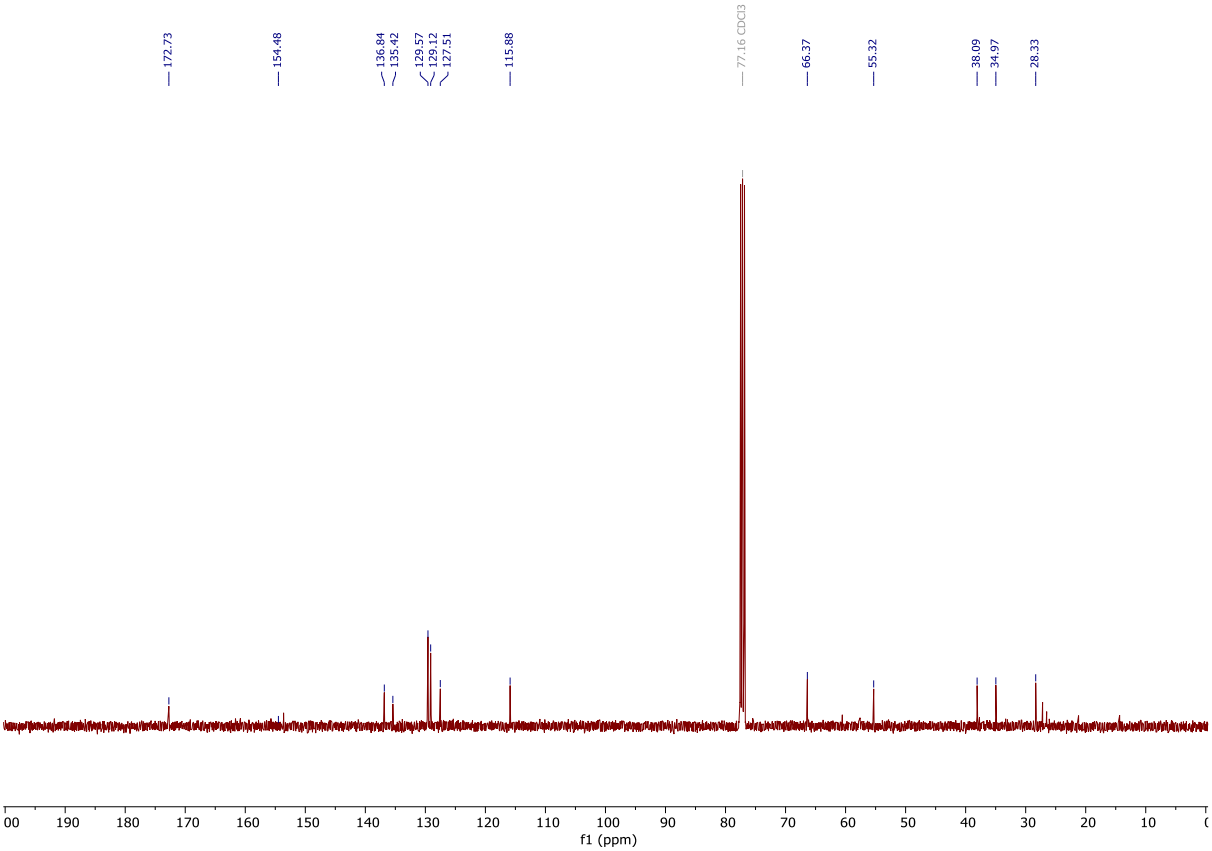
6. Appendix

6.1 Spectral Data

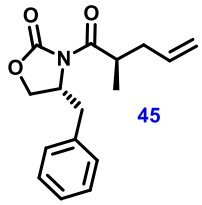
(*R*)-4-Benzyl-3-(pent-4-enoyl)oxazolidin-2-one 42







(*R*)-4-Benzyl-3-((*R*)-2-methylpent-4-enoyl)oxazolidin-2-one 45



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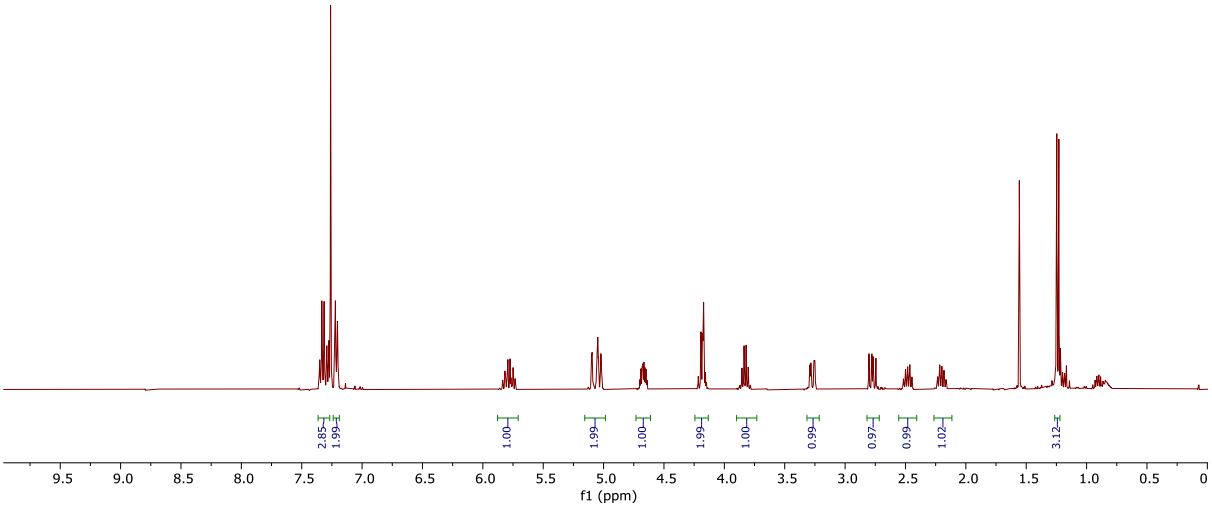
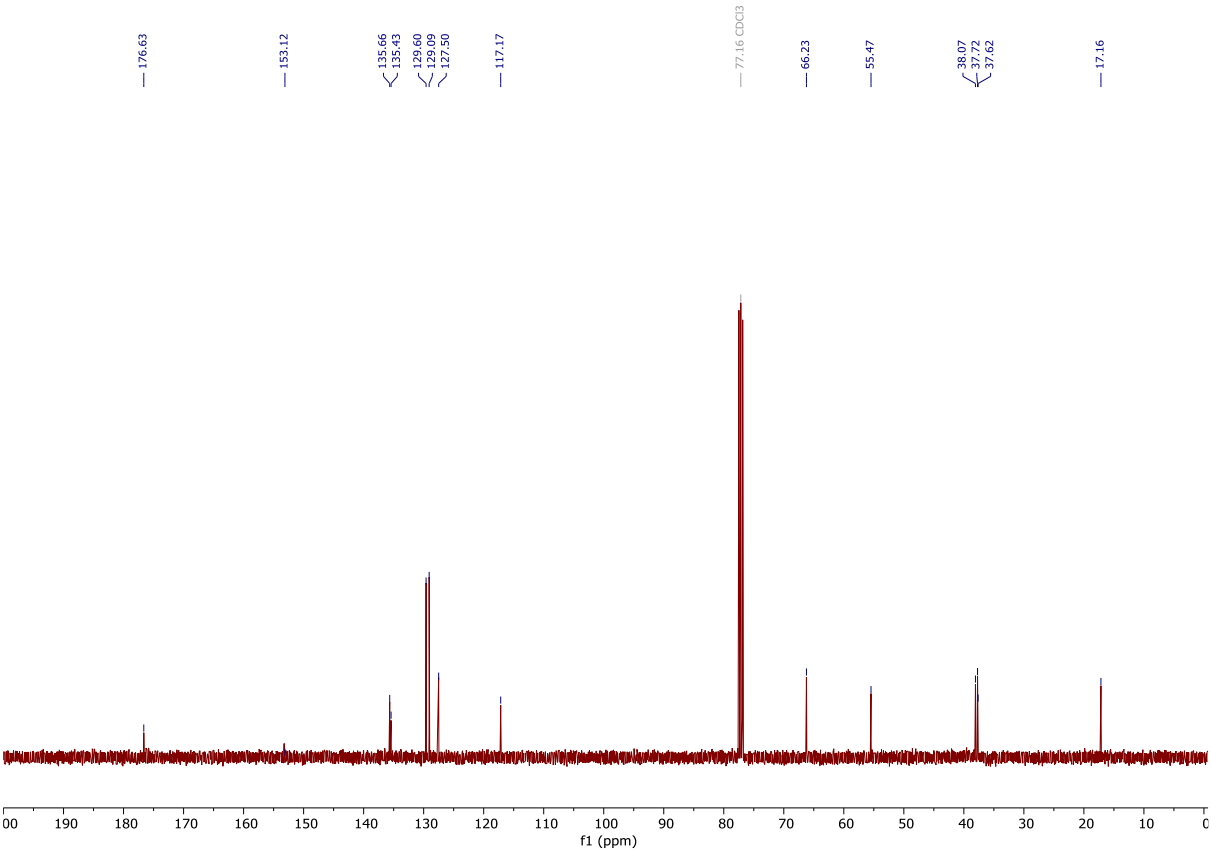
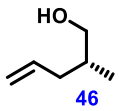
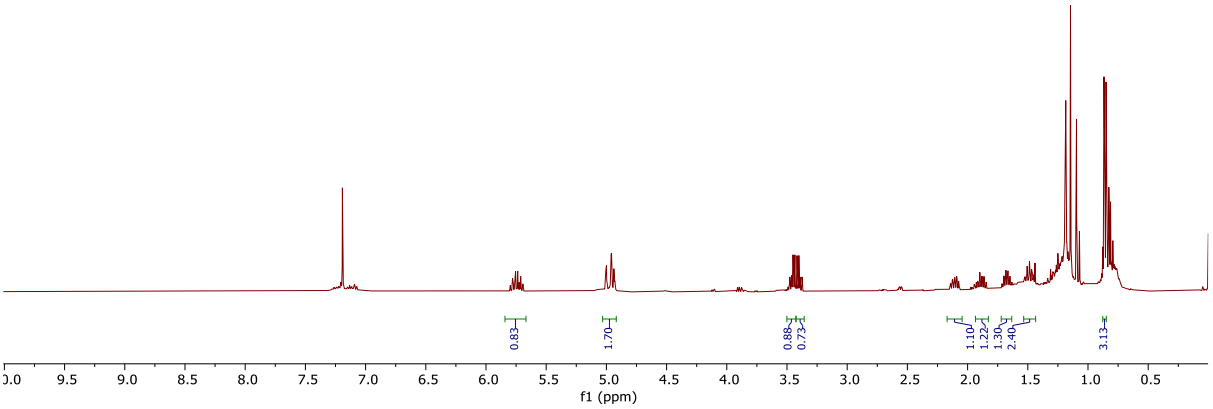
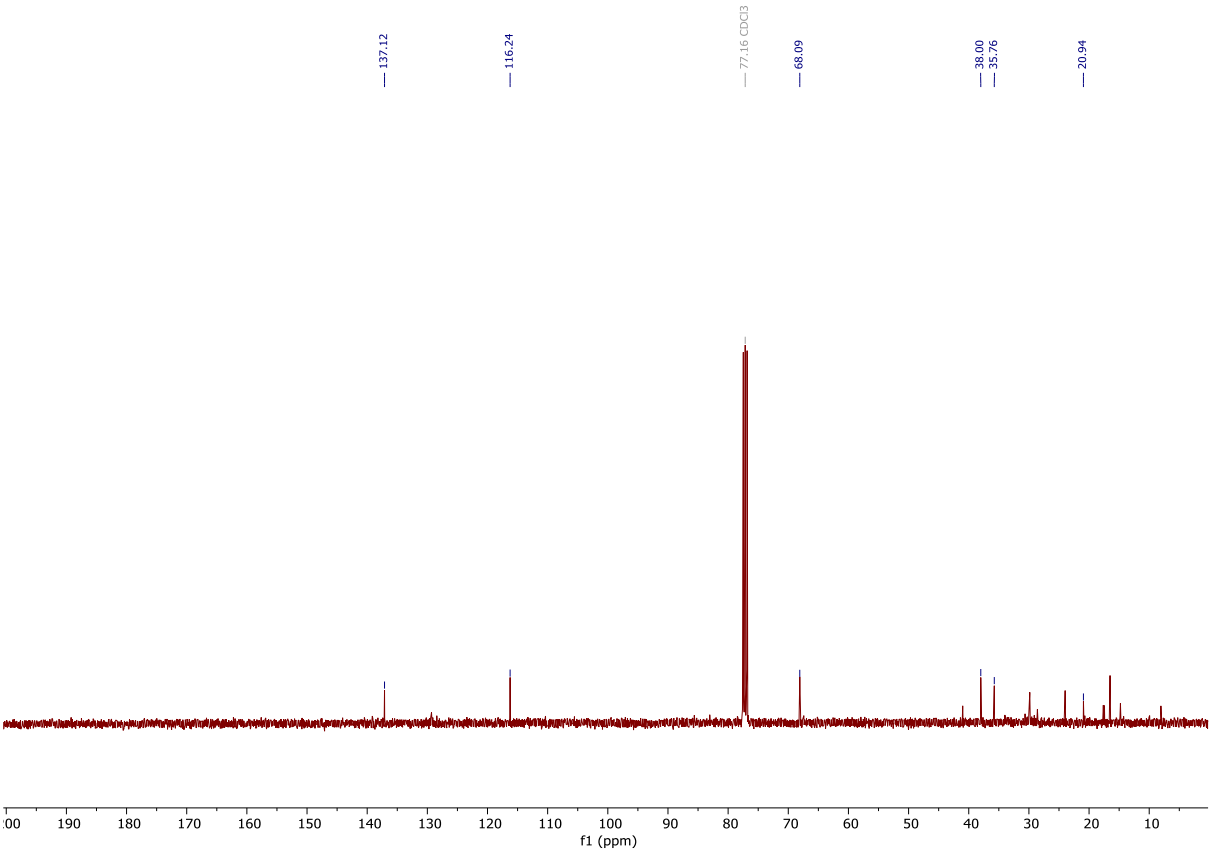




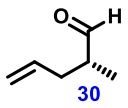
(*R*)-2-Methylpent-4-en-1-ol 46

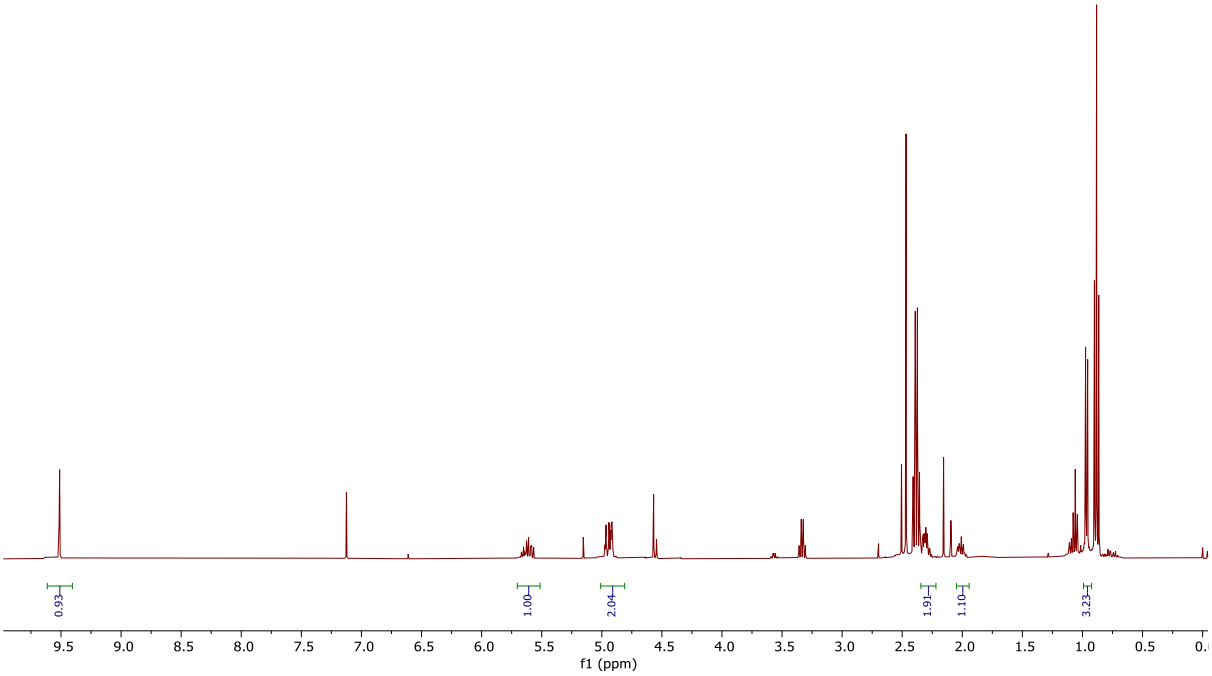


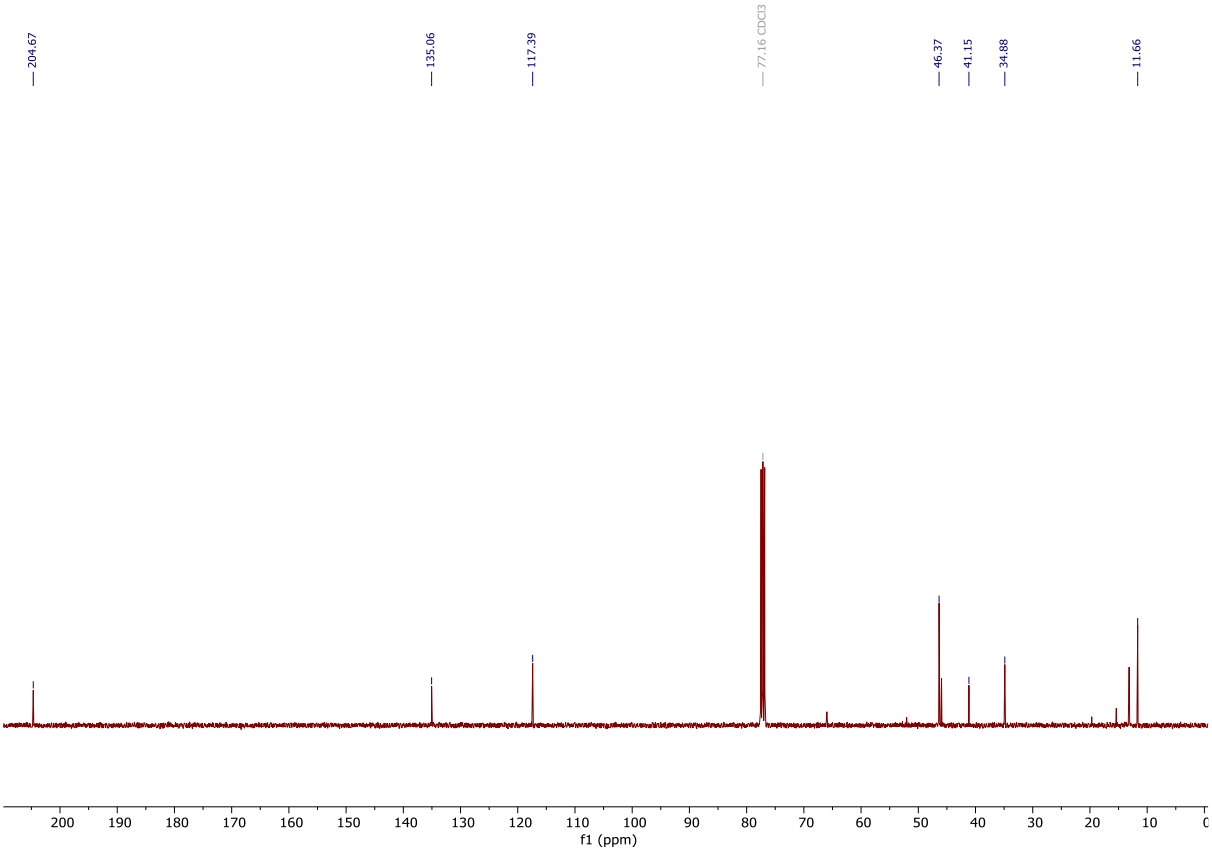




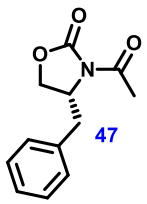
(*R*)-2-Methylpent-4-enal 30

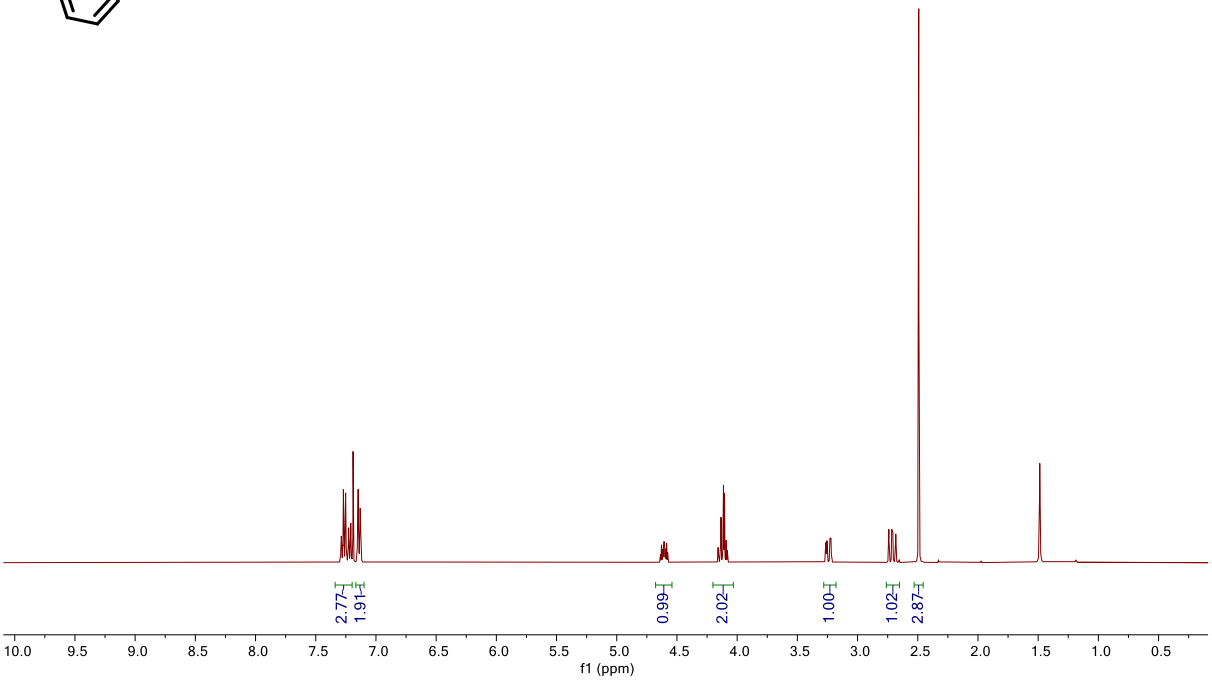


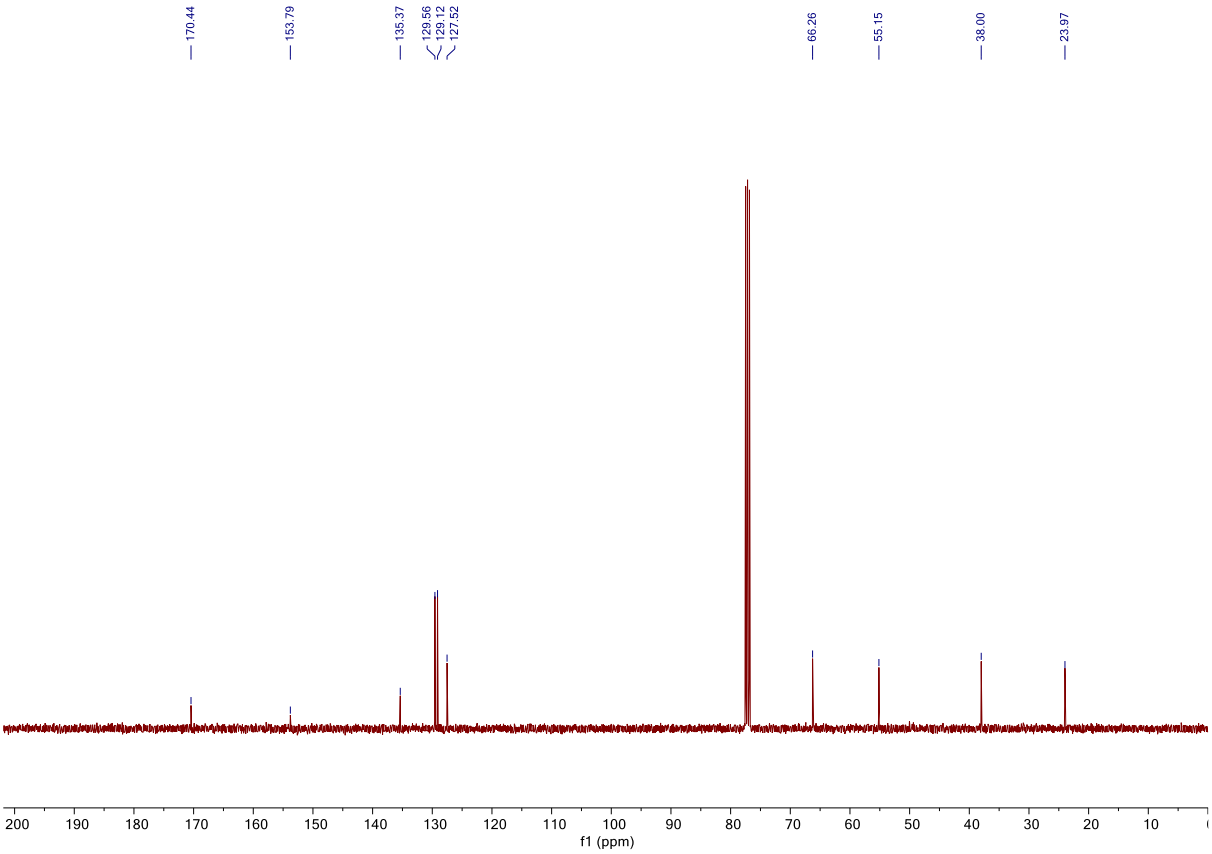




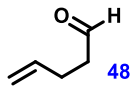
(*R*)-3-Acetyl-4-benzyloxazolidin-2-one 47

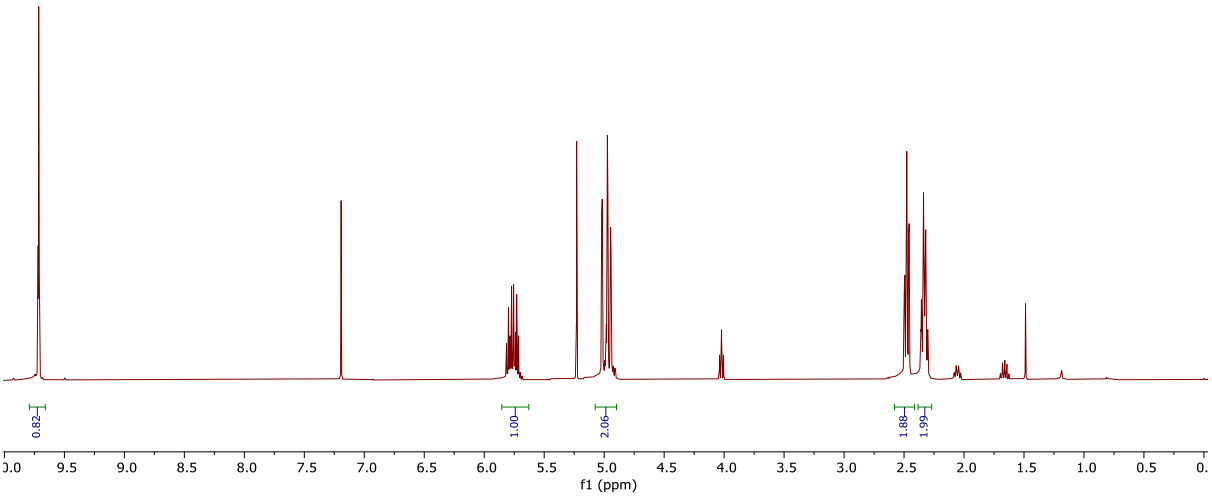


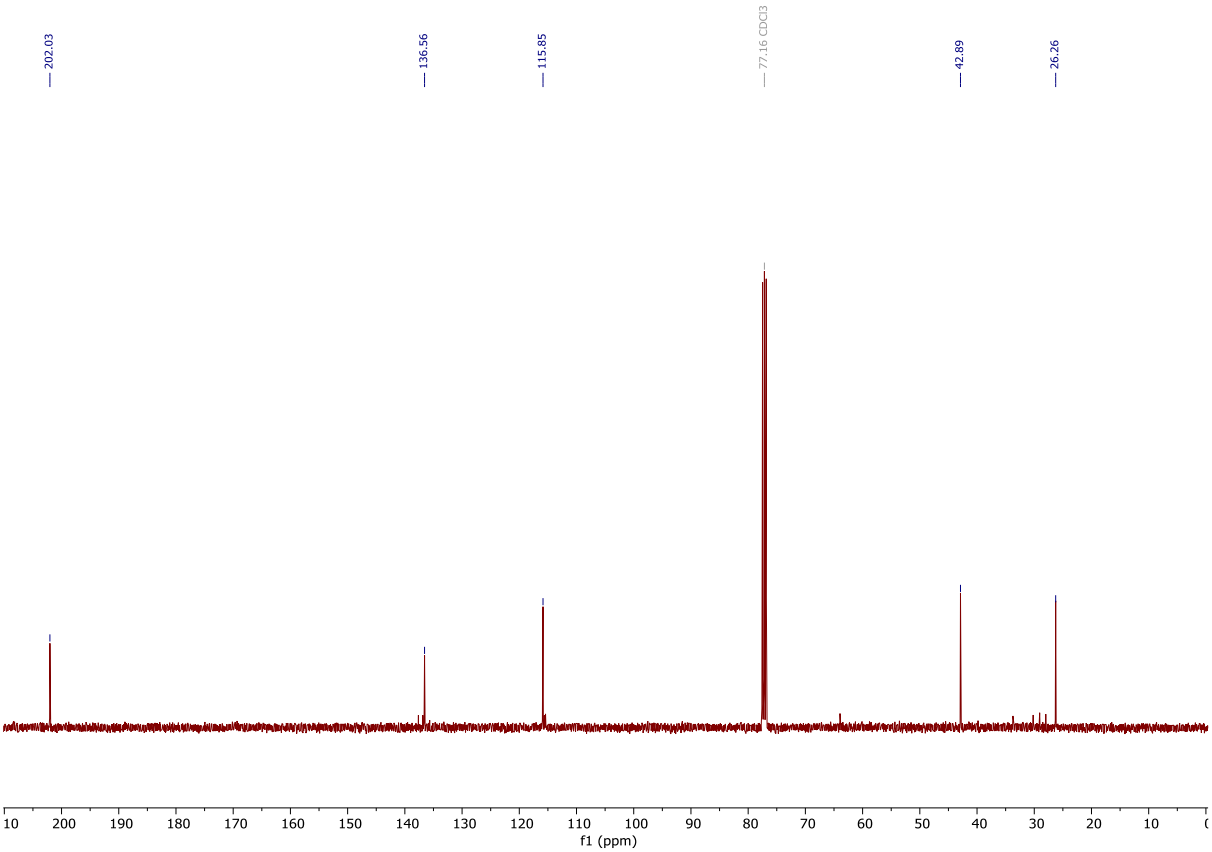




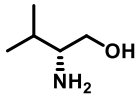
Pent-4-enal 48

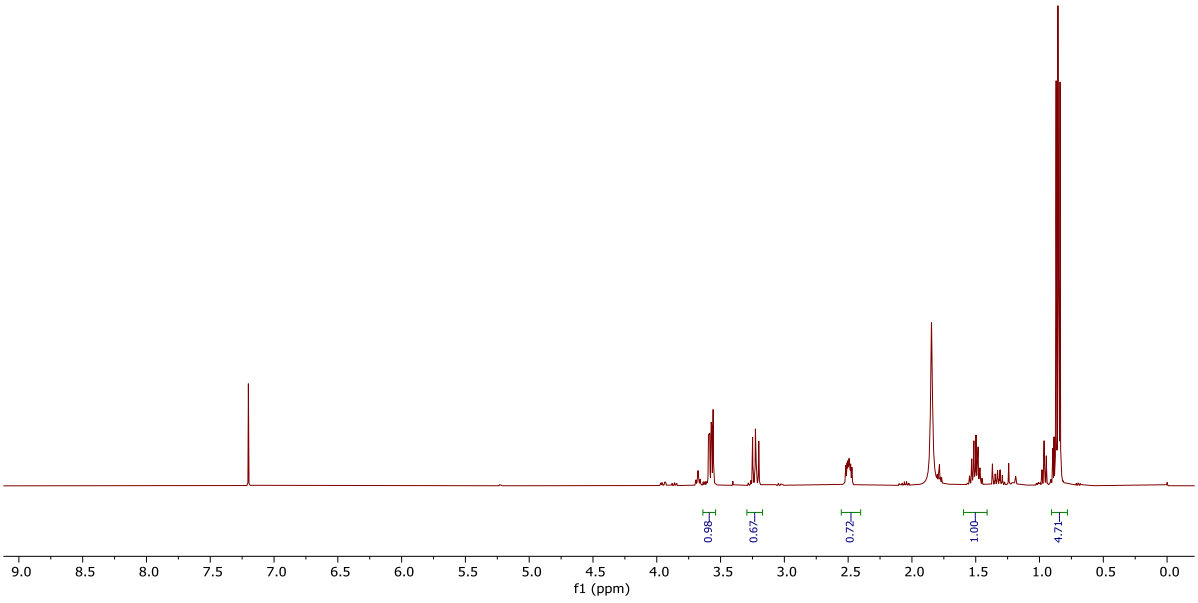




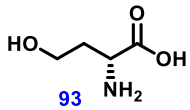


D-valinol



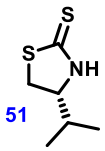


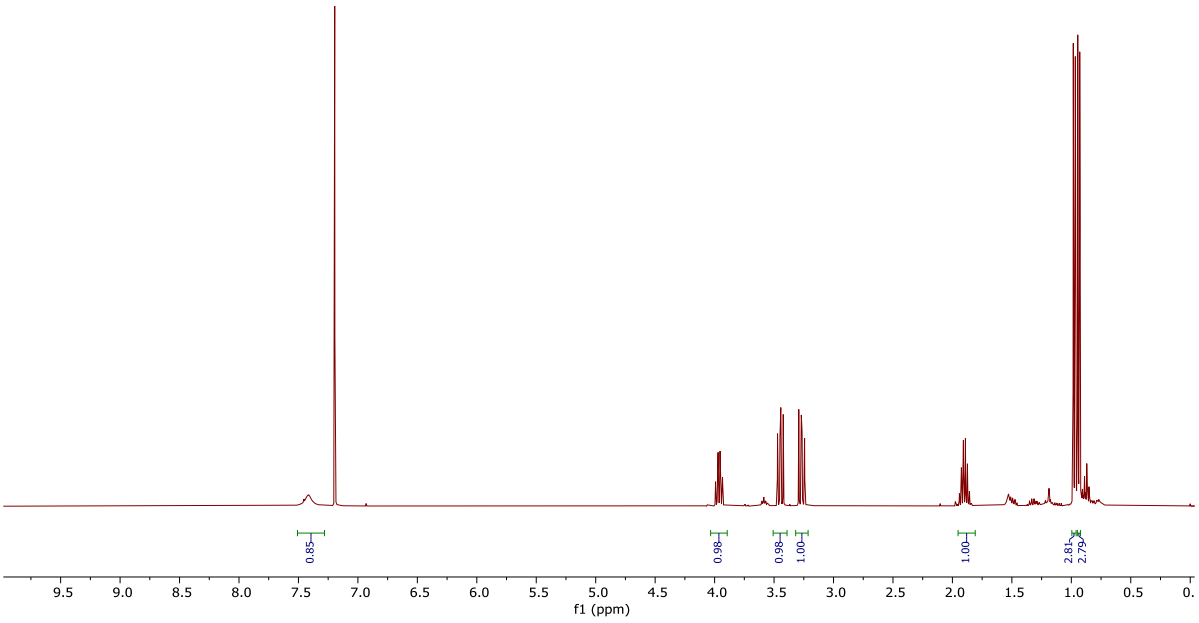
D-homoserine 93

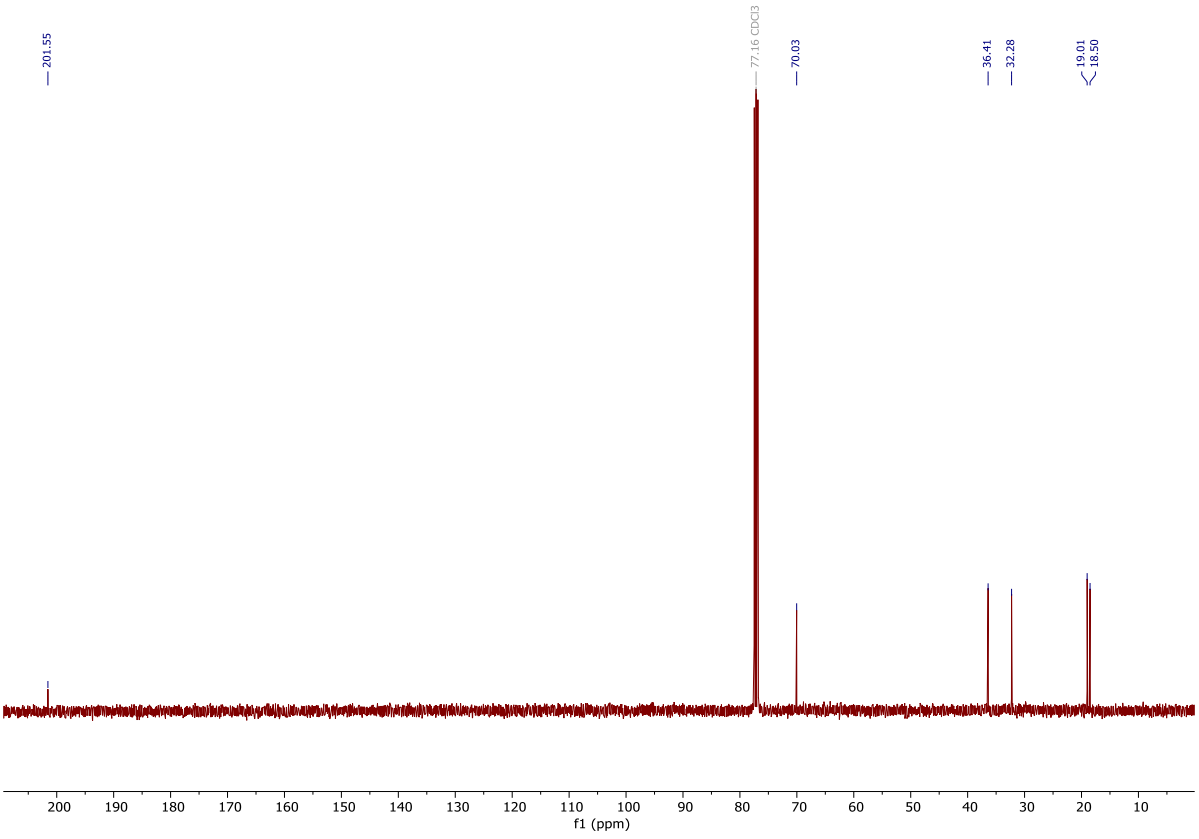


93

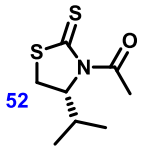
(*R*)-4-Isopropylthiazolidine-2-thione 51

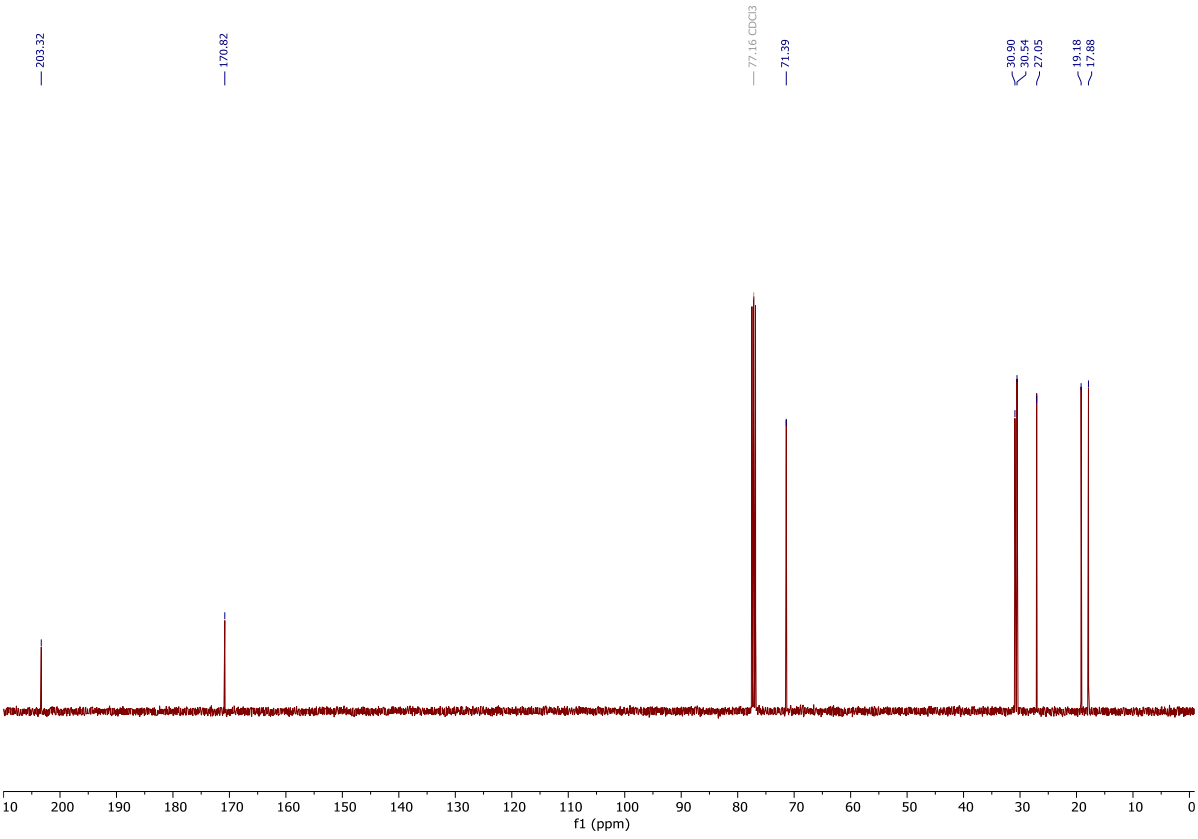




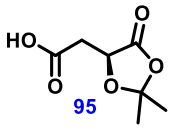


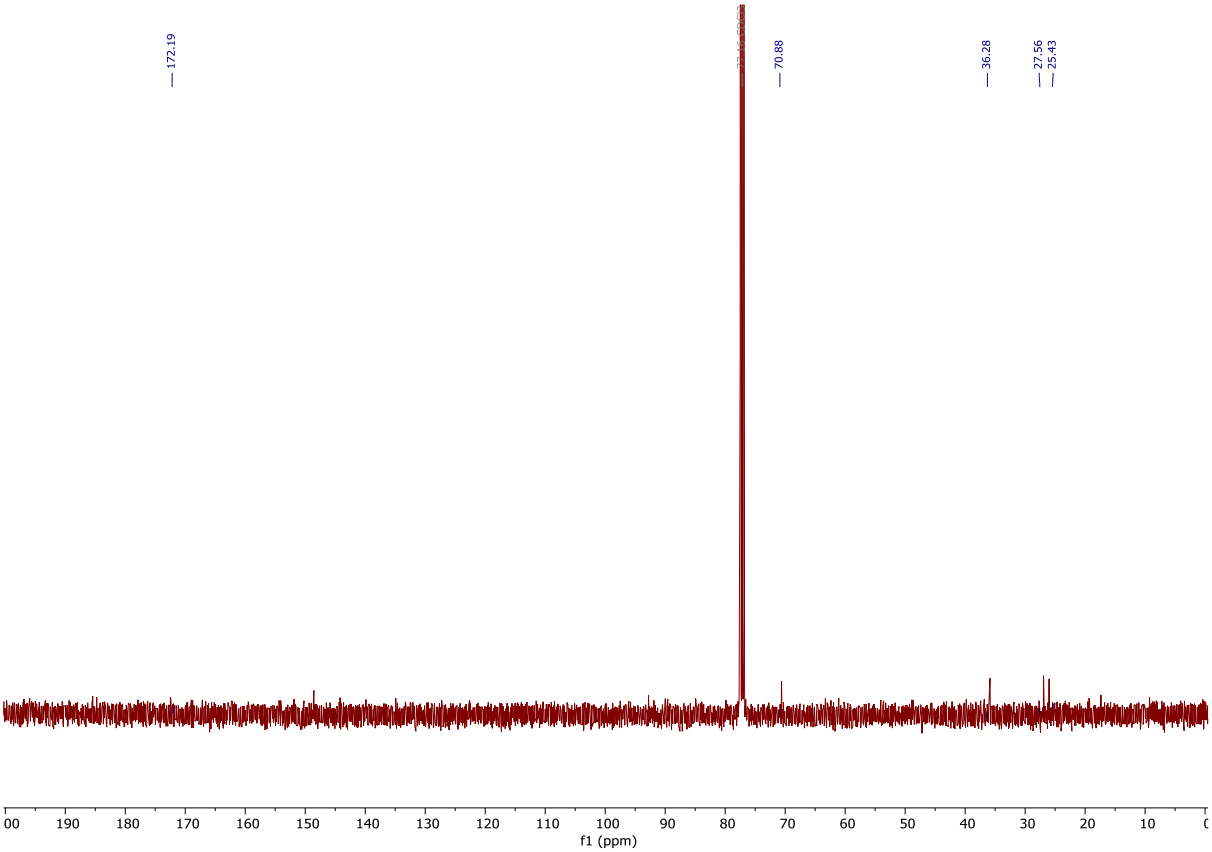
(*R*)-1-(4-Isopropyl-2-thioxothiazolidin-3-yl)ethan-1-one 52



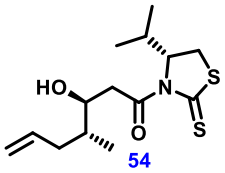


(*S*)-2-(2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl)acetic acid 95





(3*S*,4*R*)-3-Hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)-4-methylhept-6-en-1-one 54



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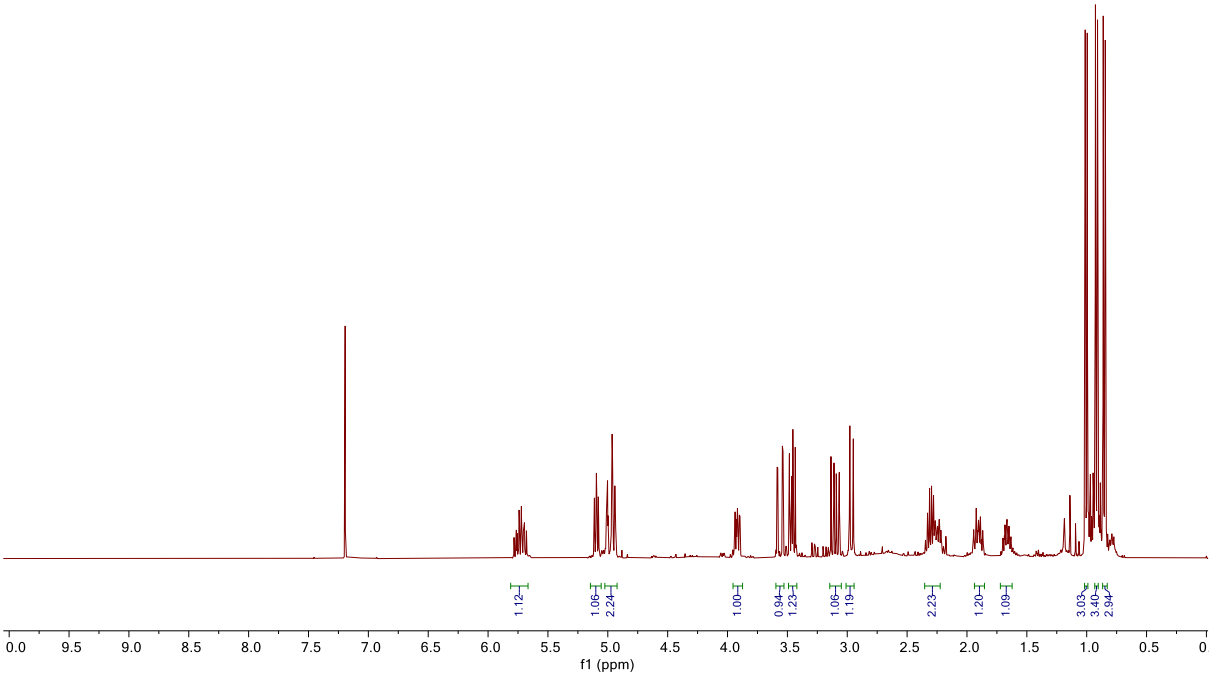
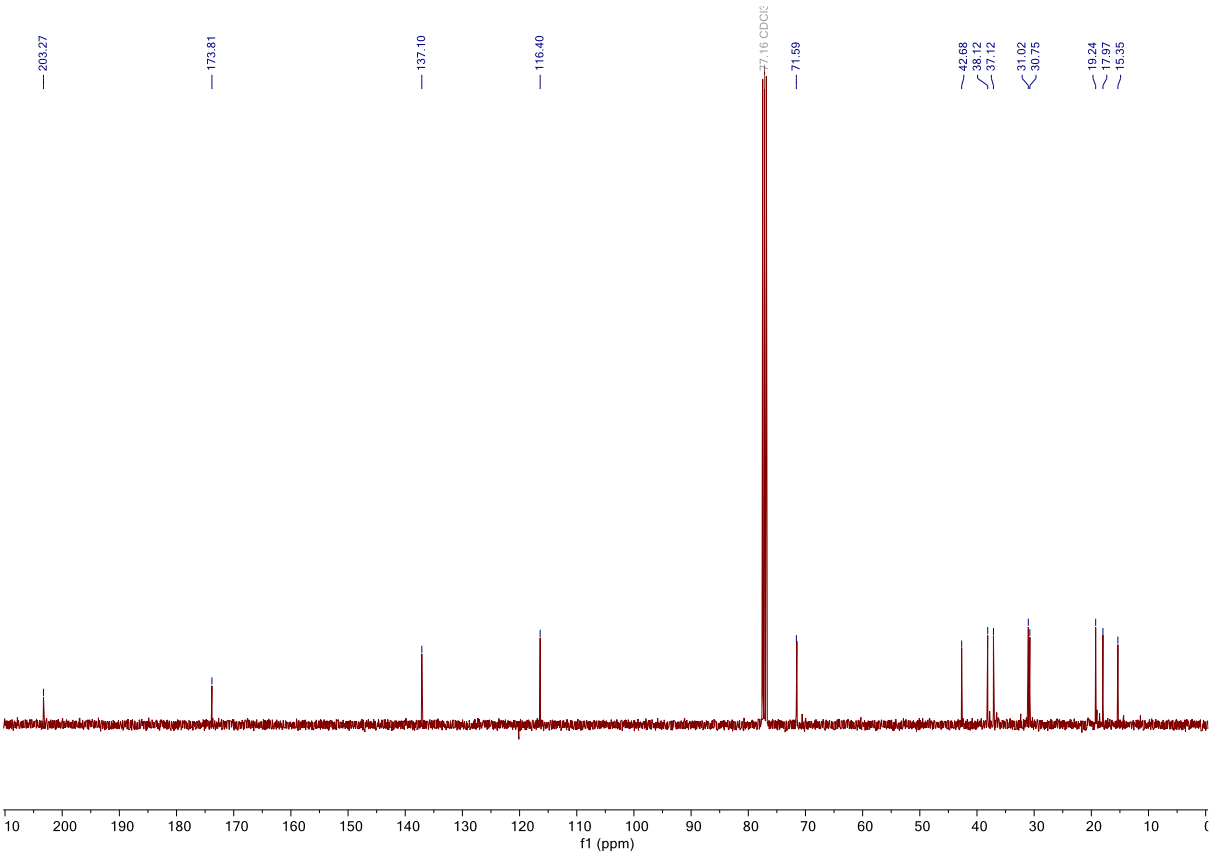
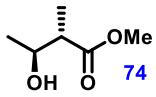
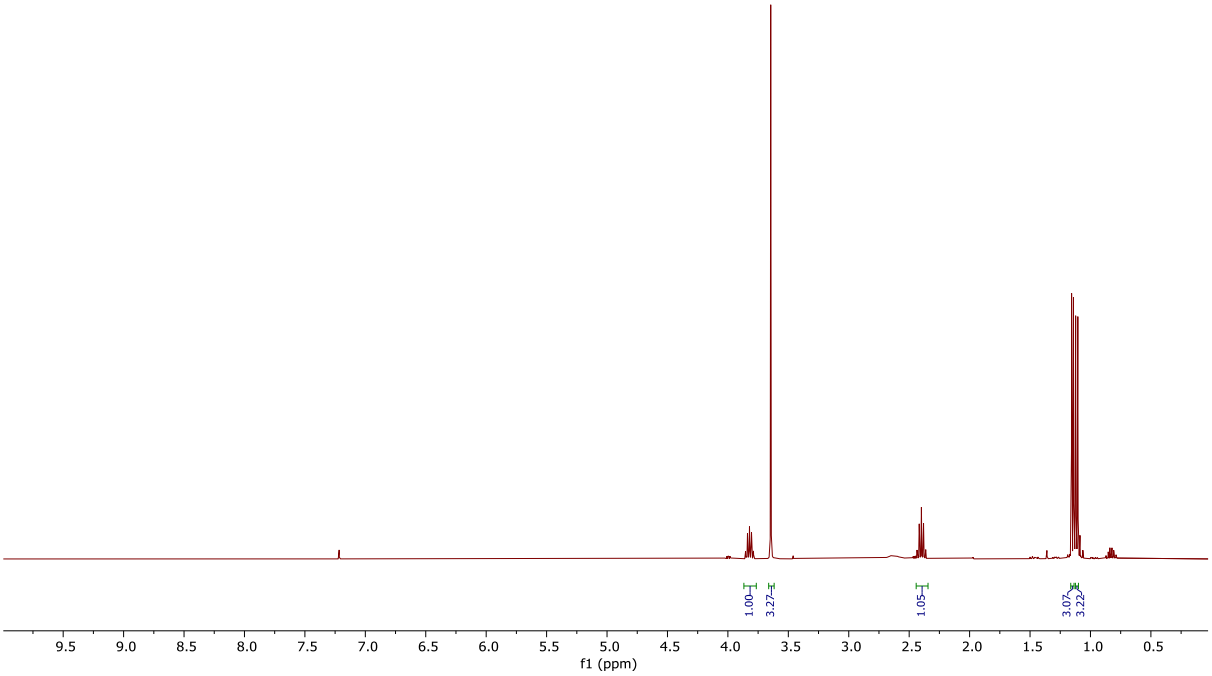
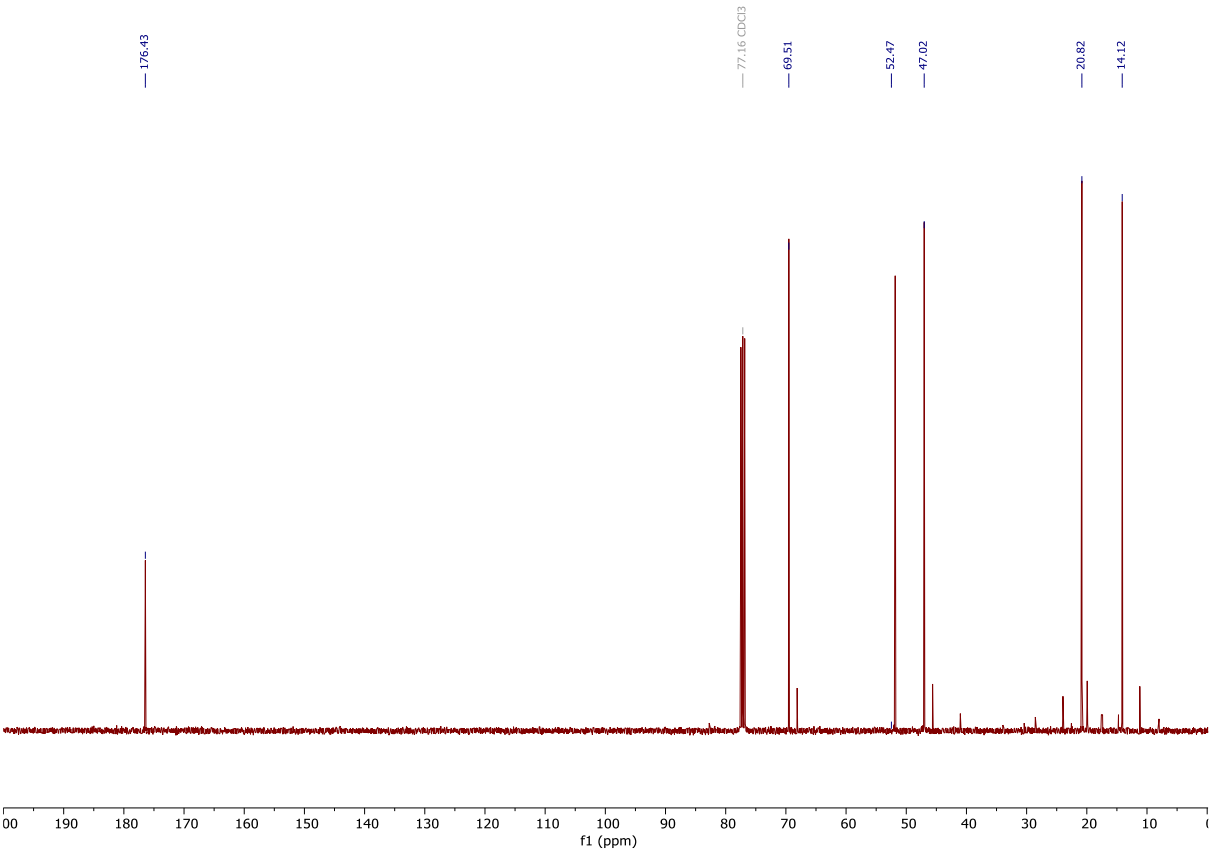




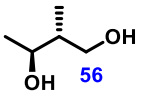
Methyl (2*S*,3*S*)-3-hydroxy-2-methylbutanoate 74

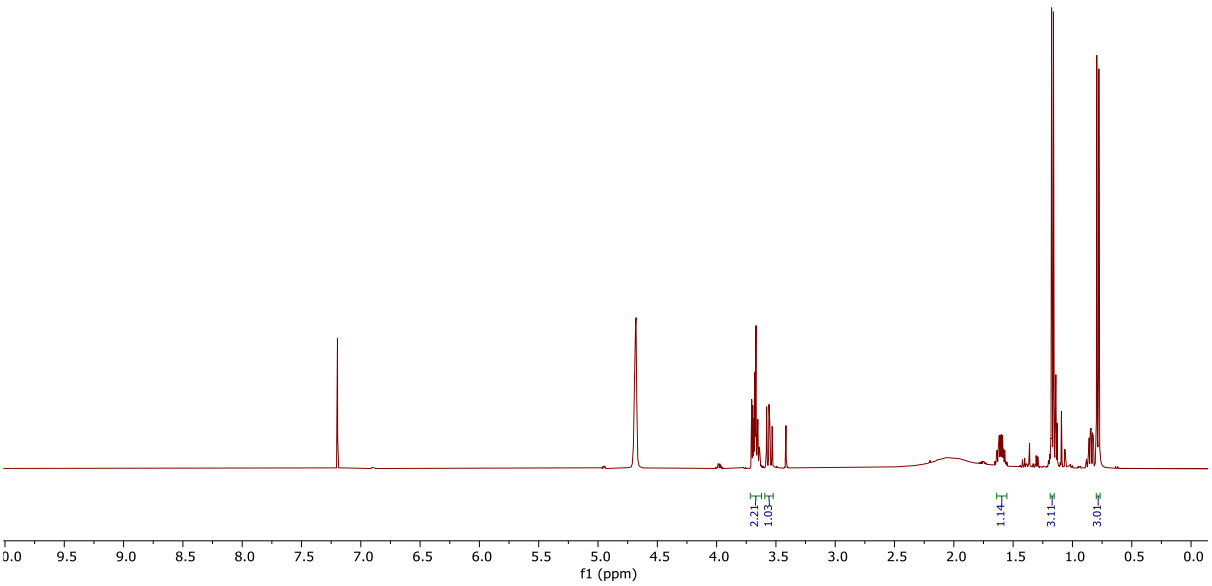


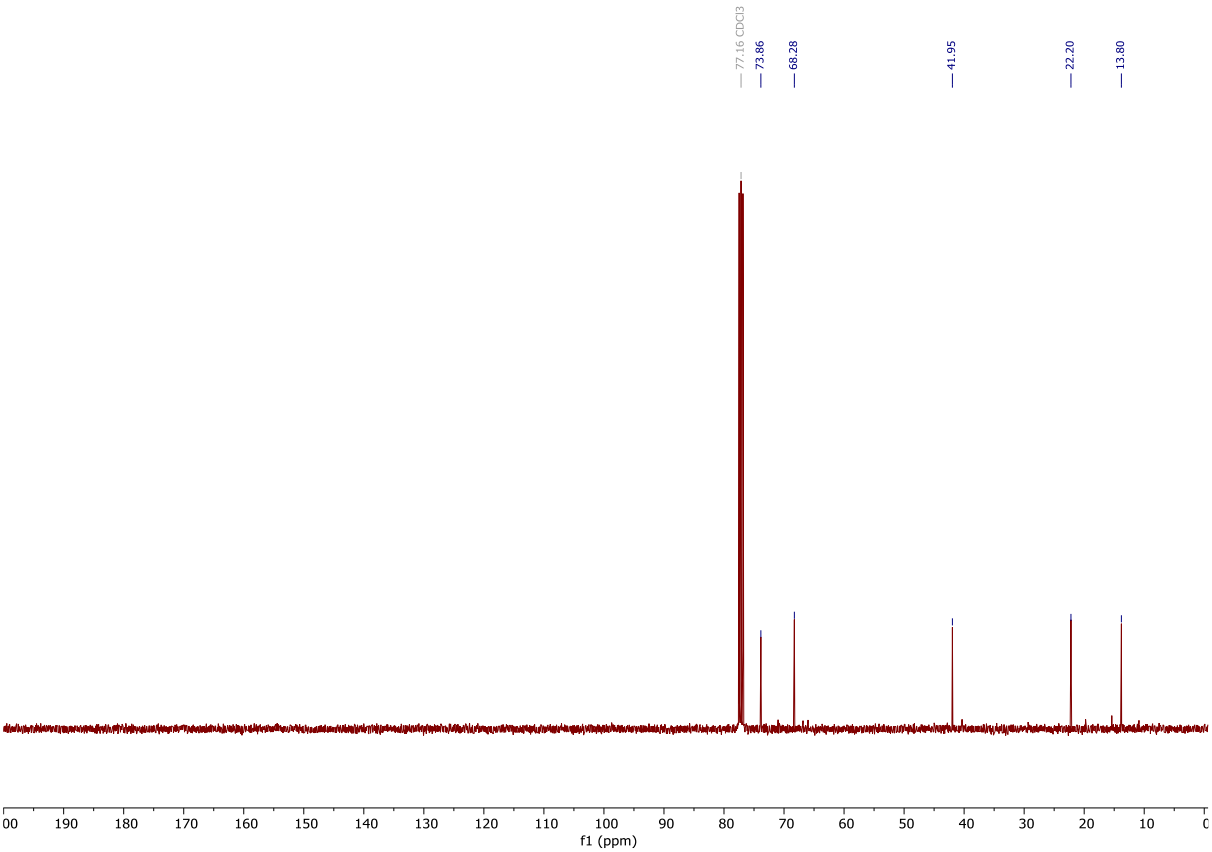




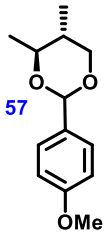
(2*R*,3*S*)-2-Methylbutane-1,3-diol 56

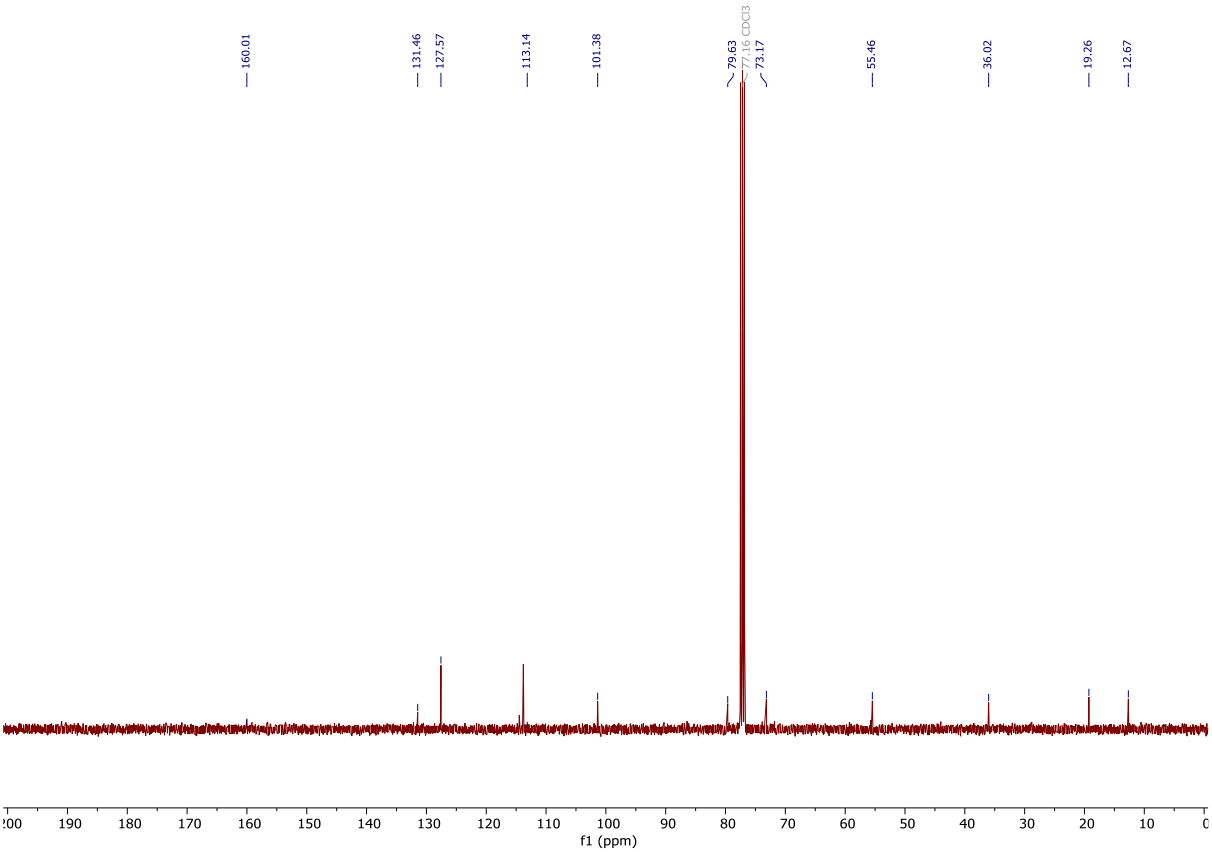




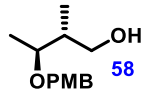


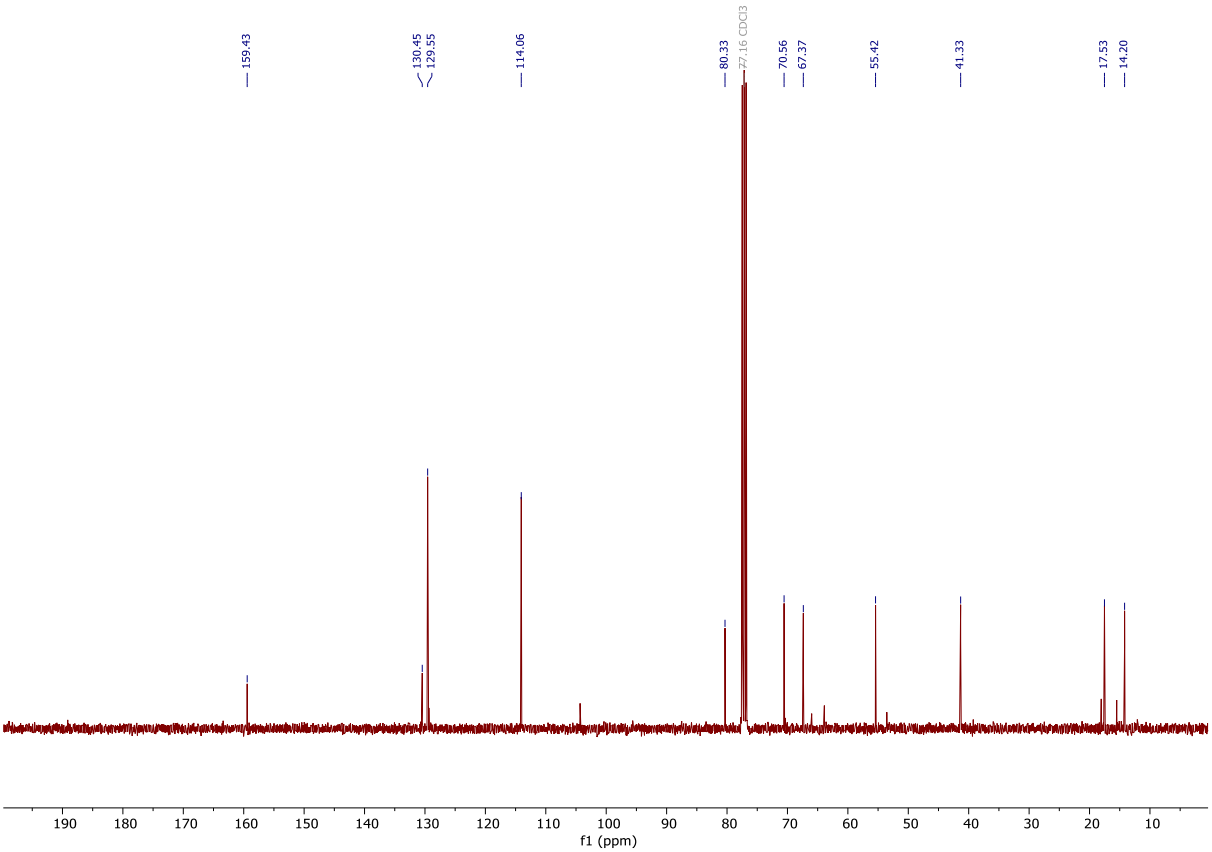
(4*S*,5*R*)-2-(4-Methoxyphenyl)-4,5-dimethyl-1,3-dioxane 57



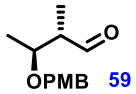


(2*R*,3*S*)-3-((4-Methoxybenzyl)oxy)-2-methylbutan-1-ol 58

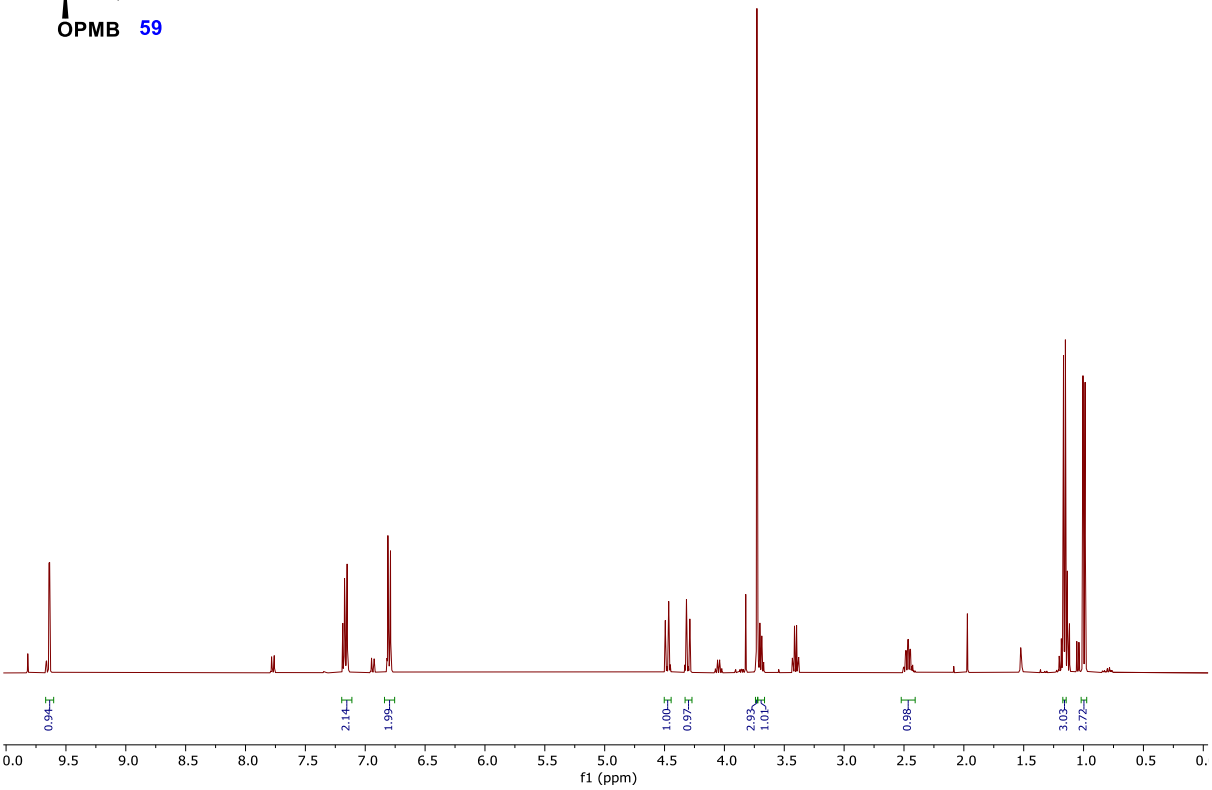


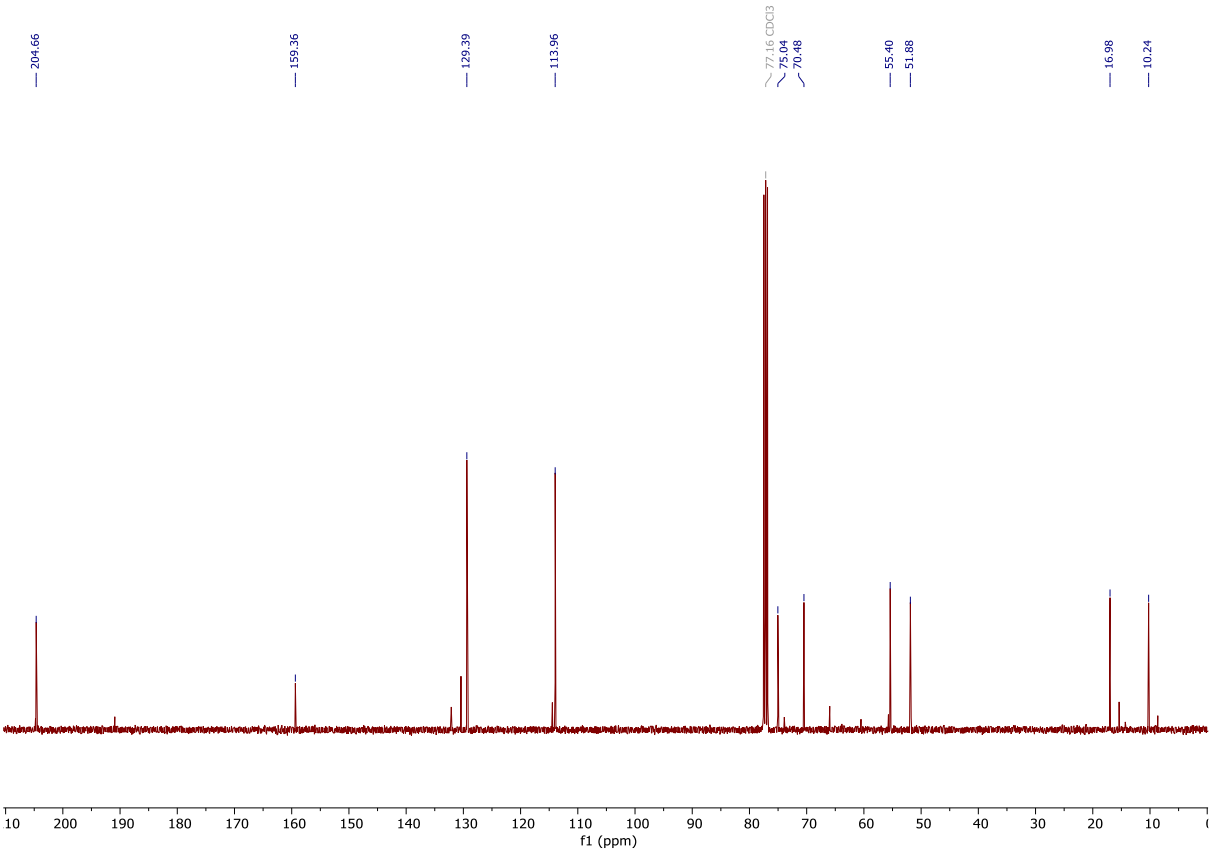


(2*S*,3*S*)-3-((4-Methoxybenzyl)oxy)-2-methylbutanal 59

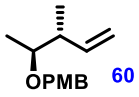


59





(2*S*,3*S*)-3-((4-Methoxybenzyl)oxy)-3-methylpent-1-ene 60



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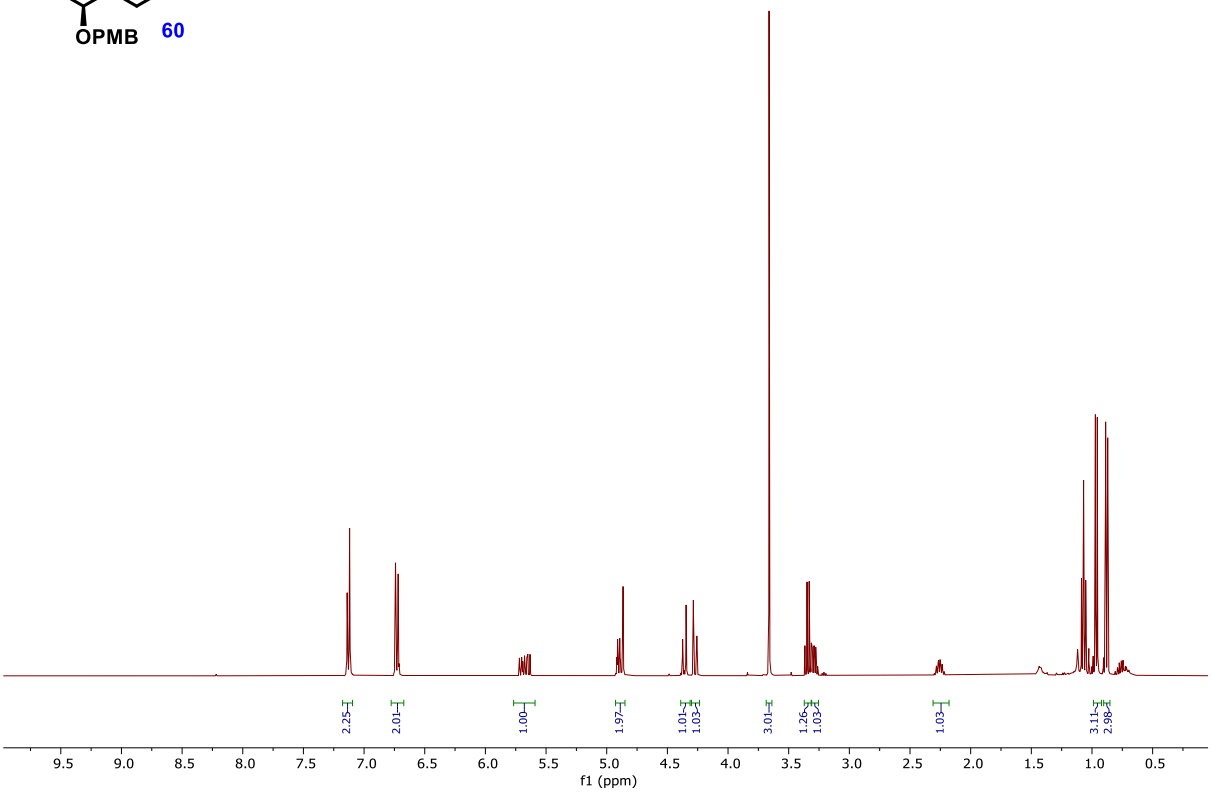
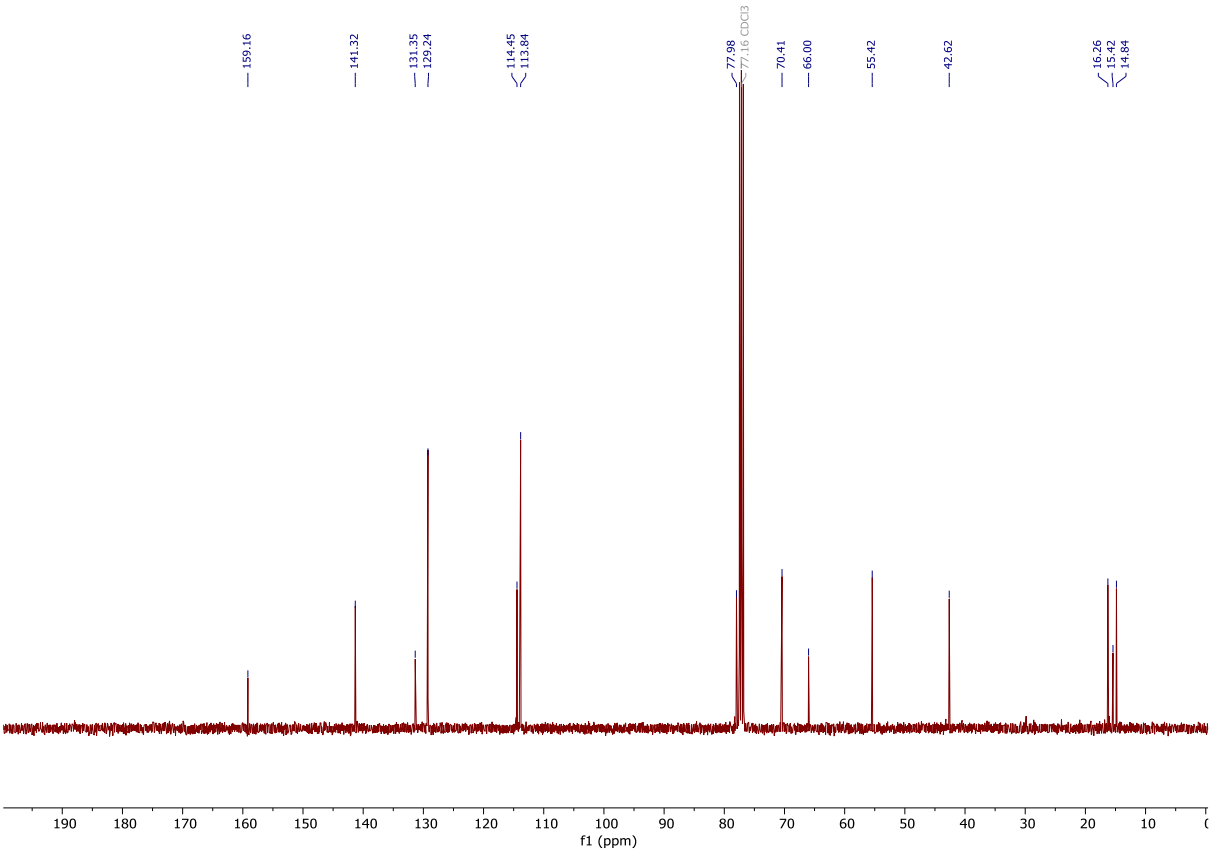
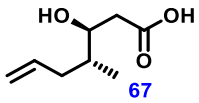
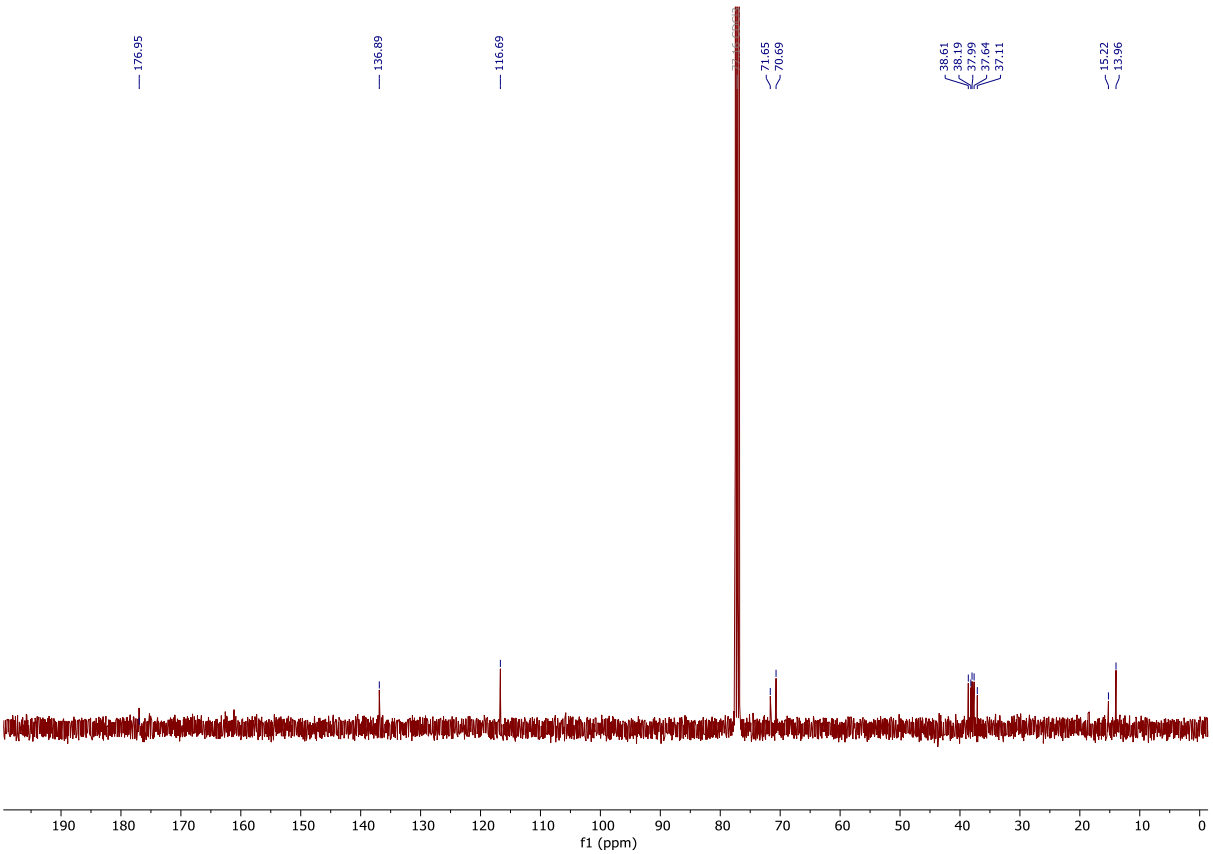




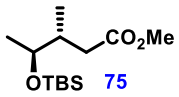
(3*S*,4*R*)-3-Hydroxy-4-methylhept-6-enoic 67



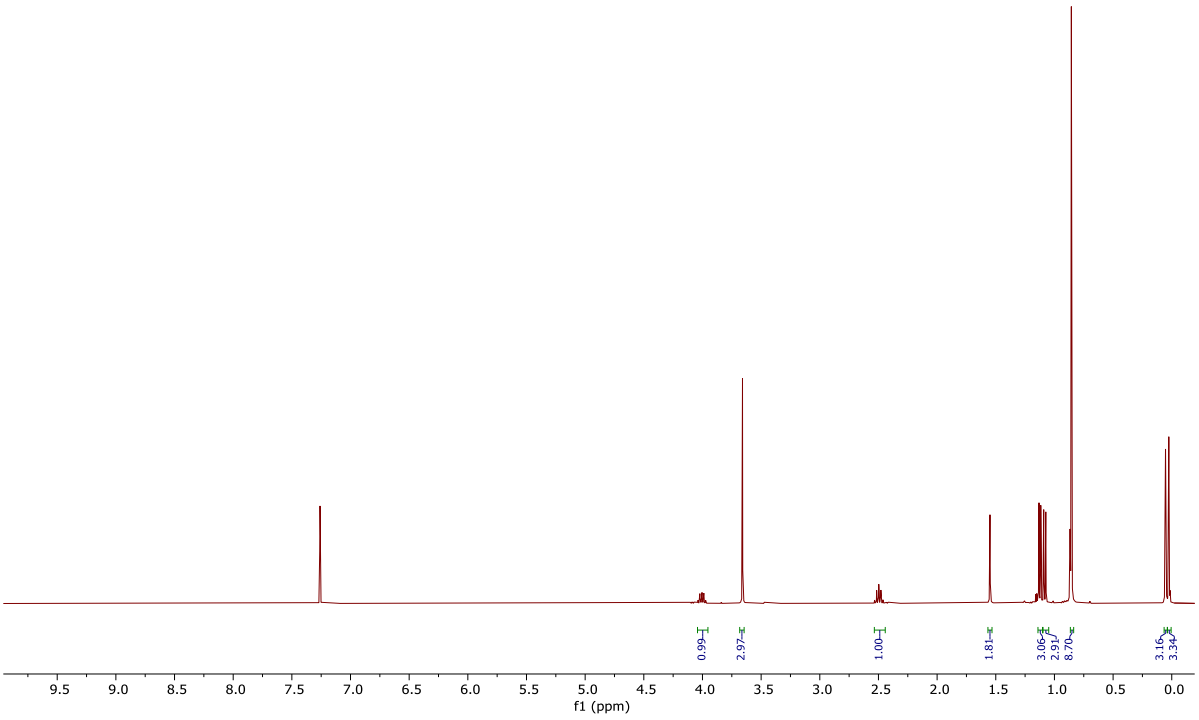
67

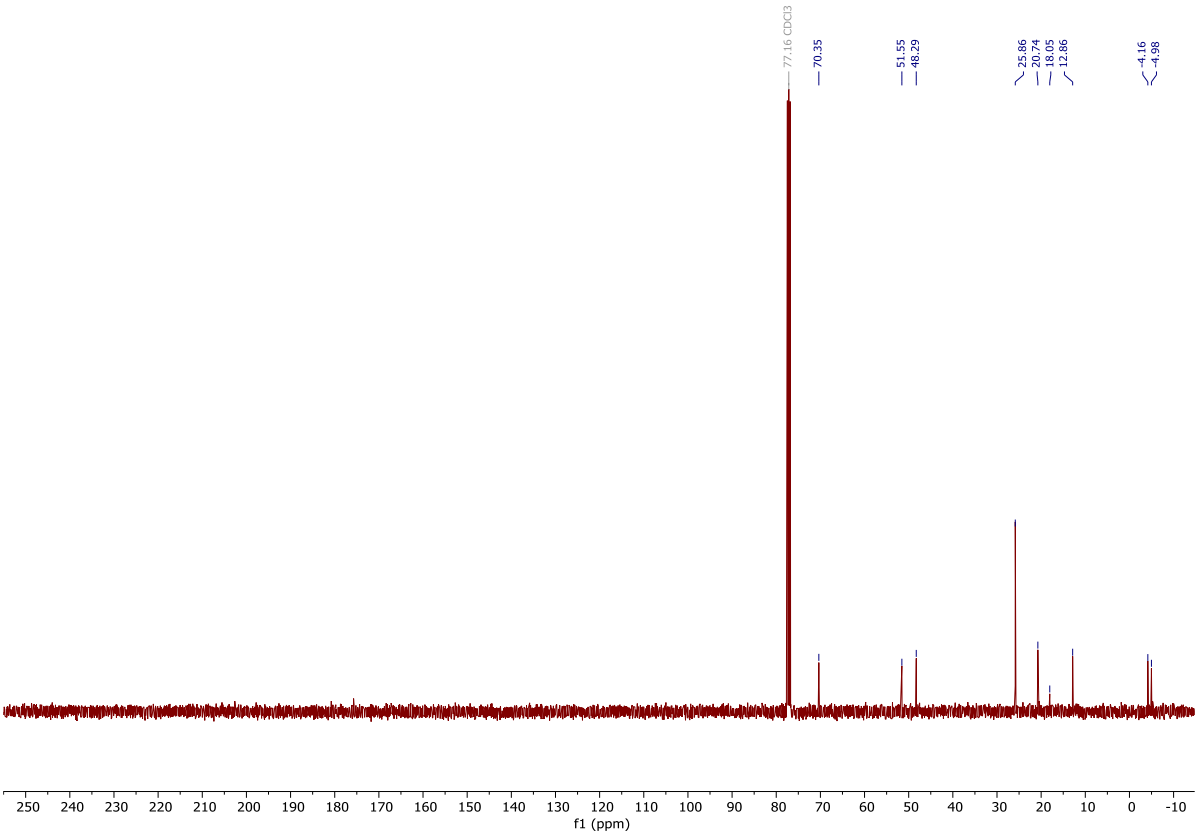


Methyl (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylbutanoate 75

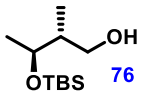


75

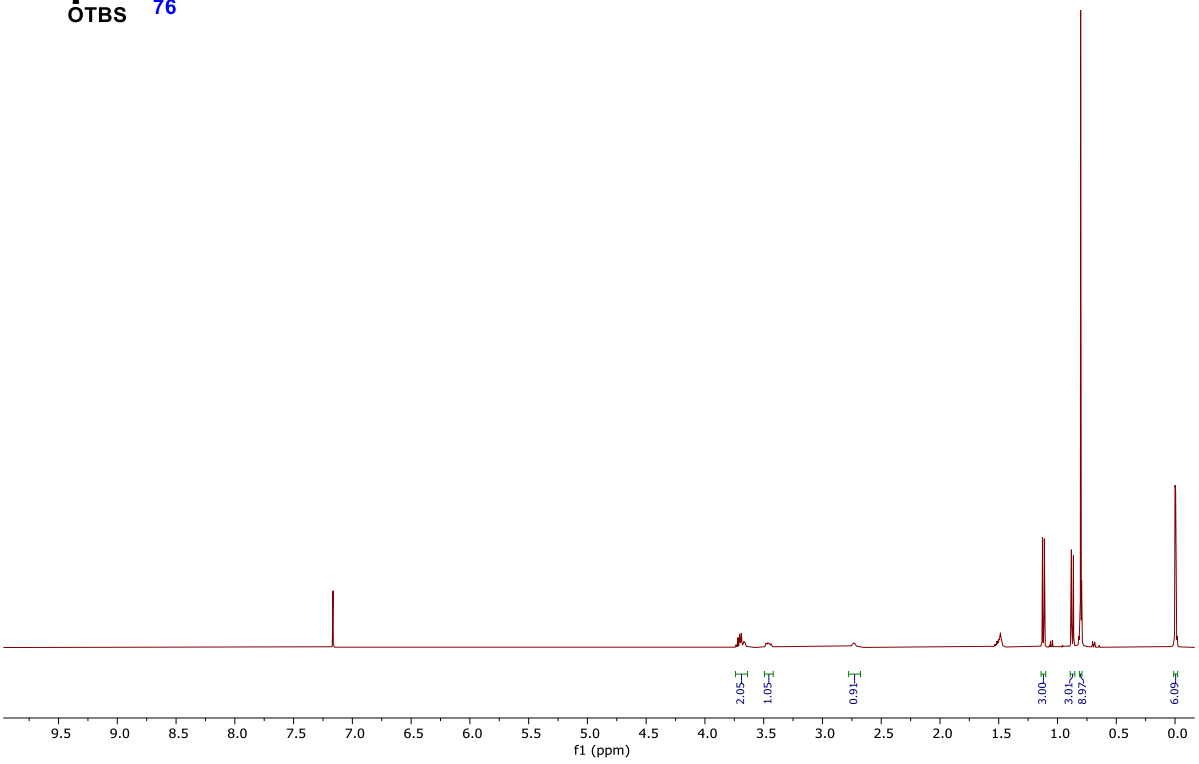


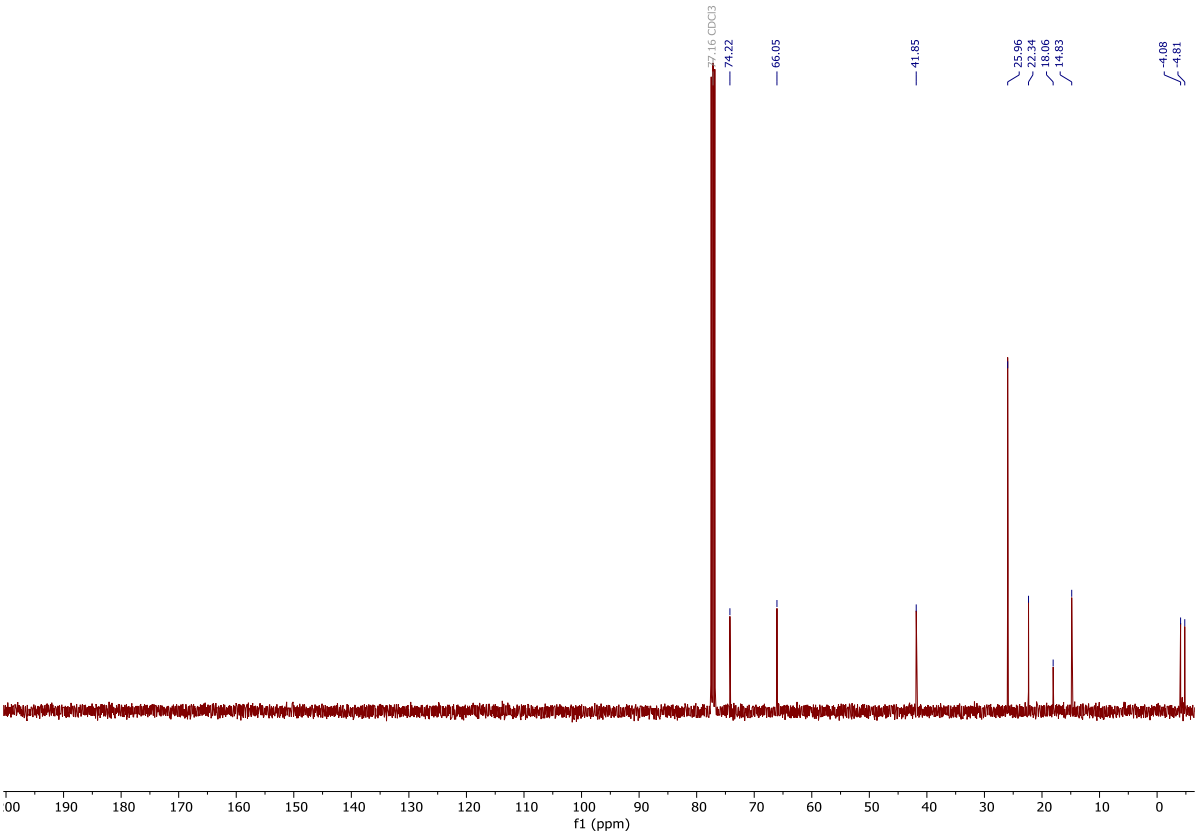


(2*R*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylbutan-1-ol 76

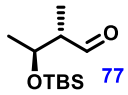


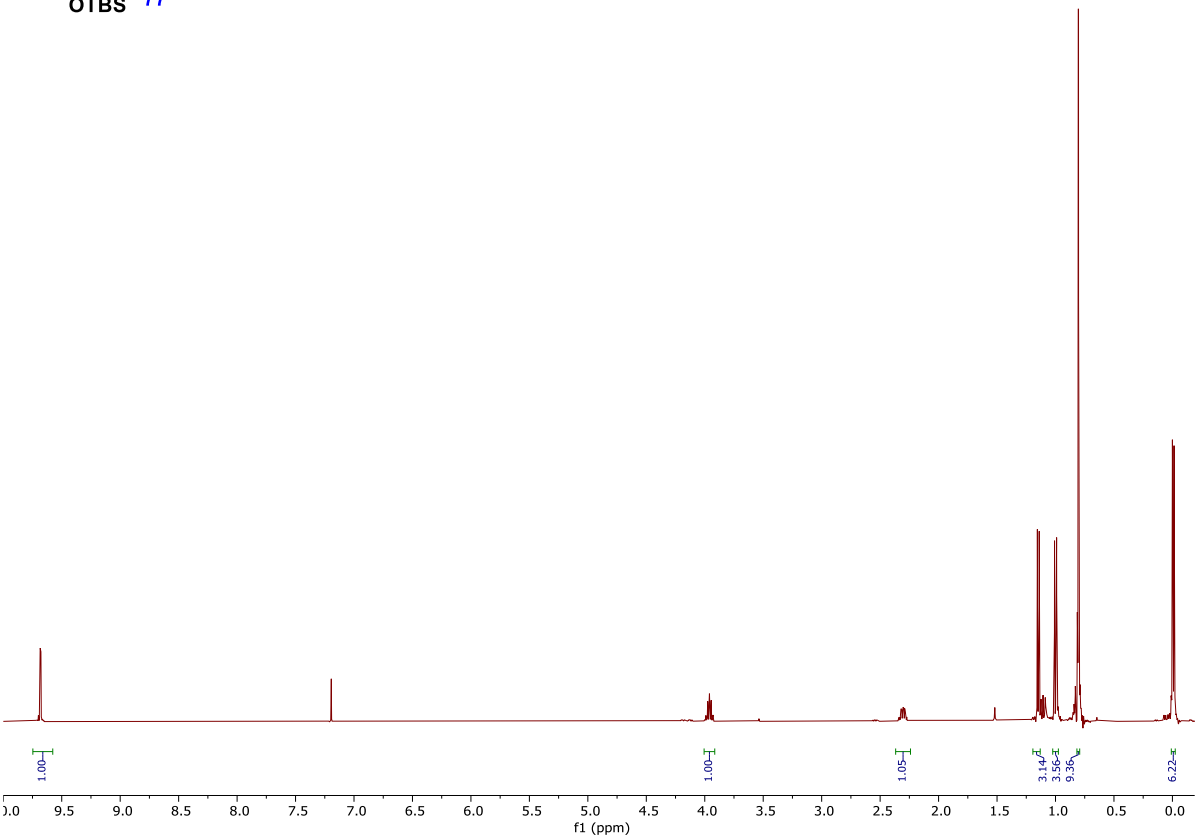
76

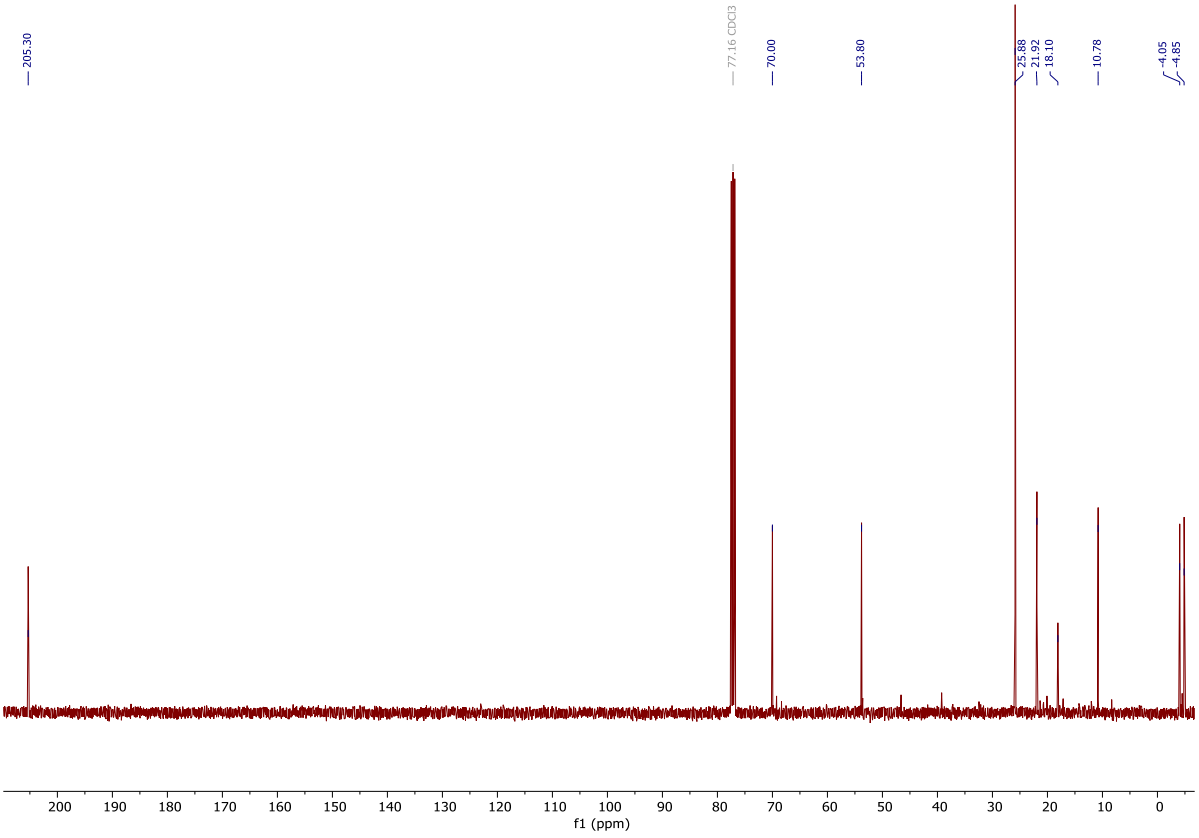




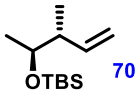
(2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylbutanal 77

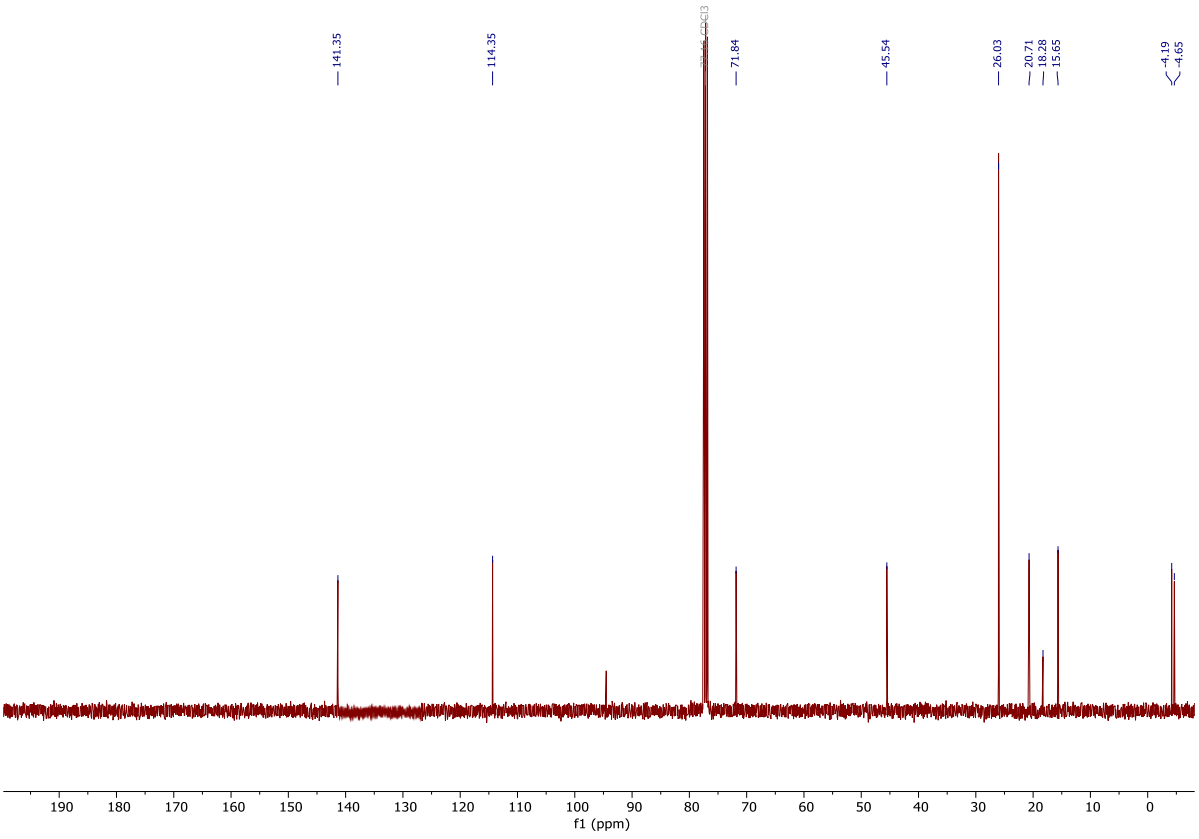




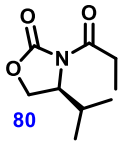


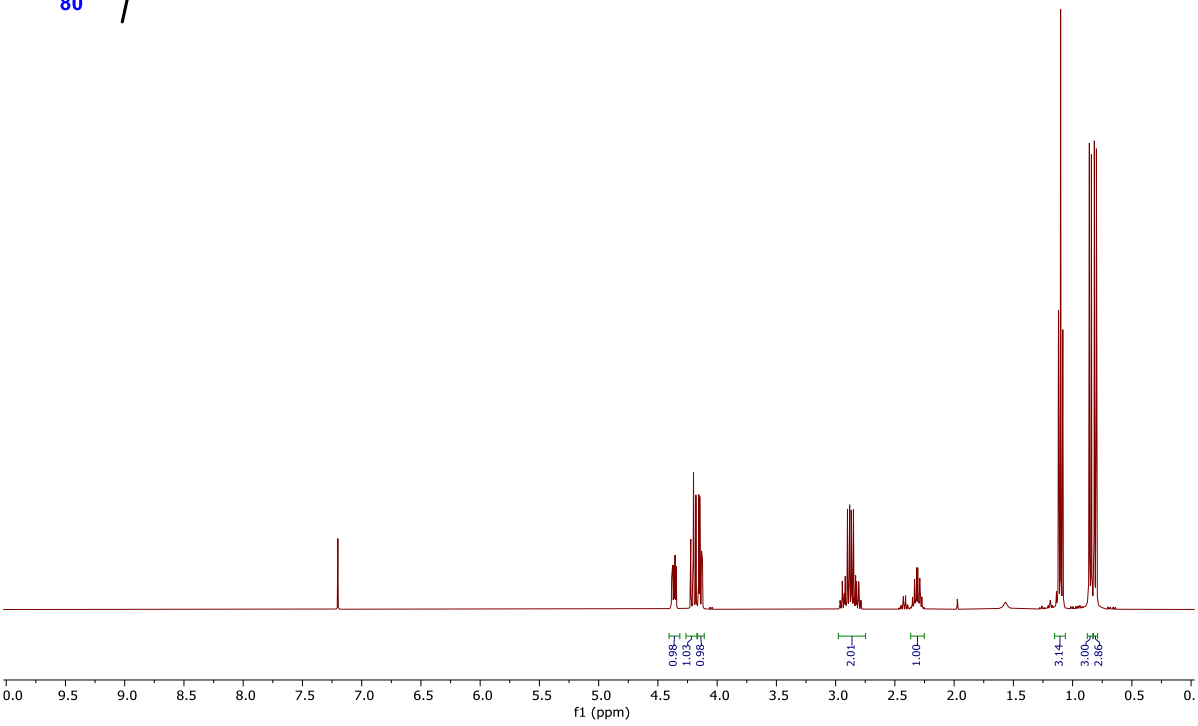
(3*R,4S*)-4-(tertbutyldimethylsilyloxy)-3-methylpent-1-ene 70

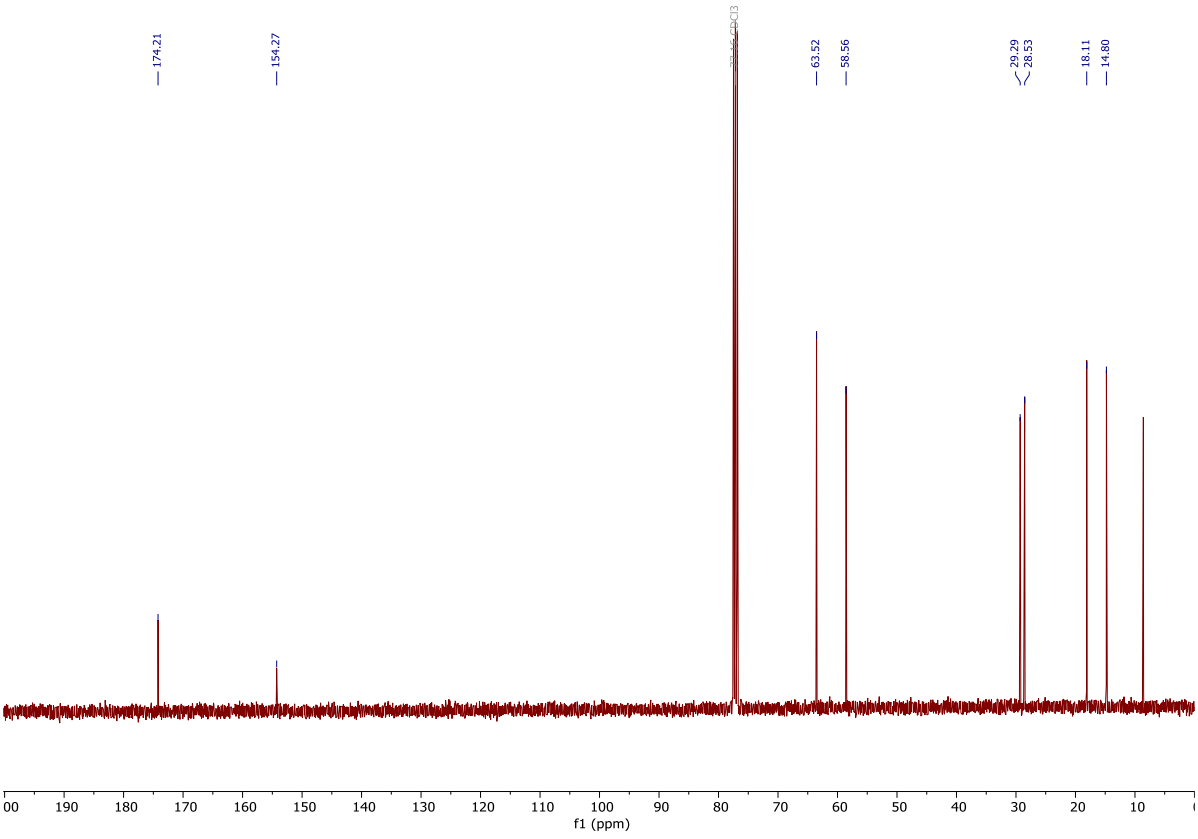




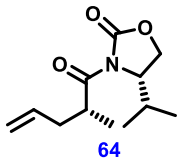
(*S*)-4-Isopropyl-3-propionyloxazolidin-2-one 80



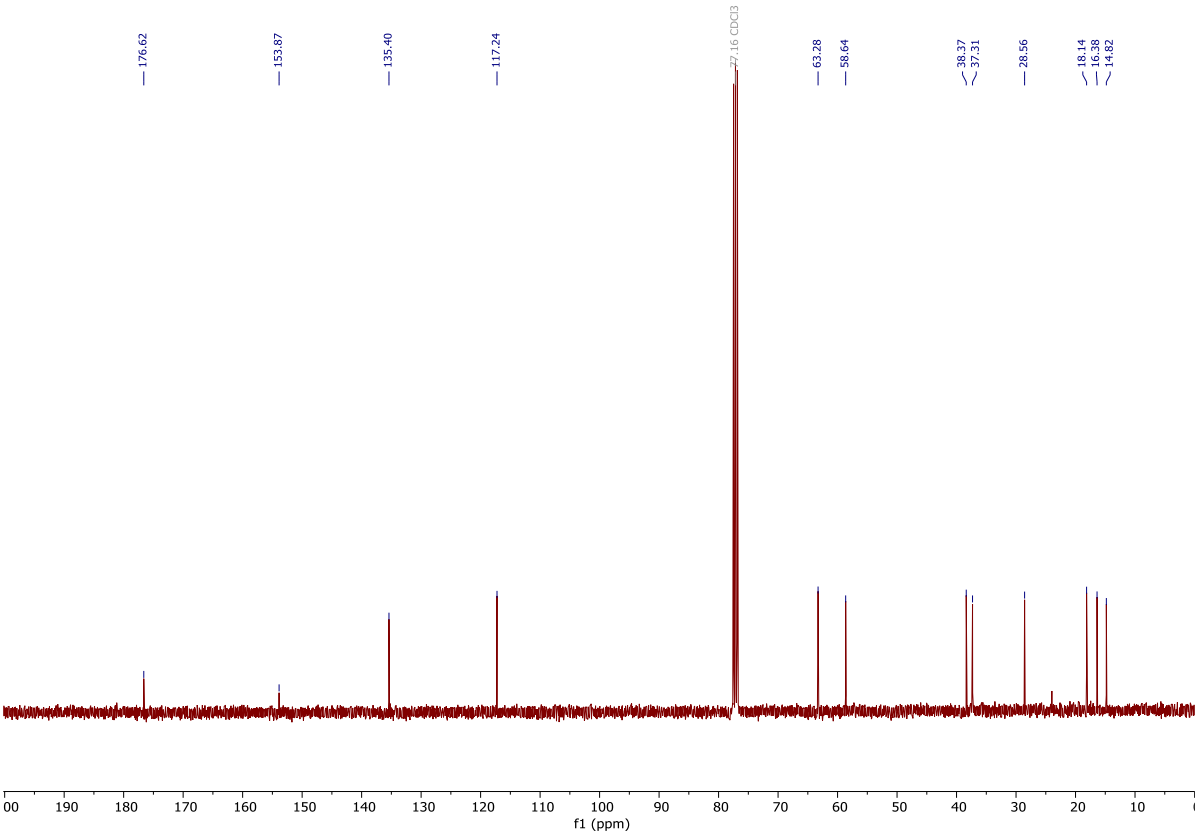




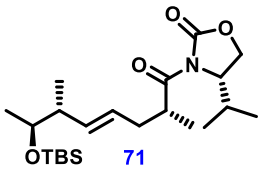
(*S*)-4-Isopropyl-3-((*R*)-2-methylpent-4-enoyl)oxazolidin-2-one 64



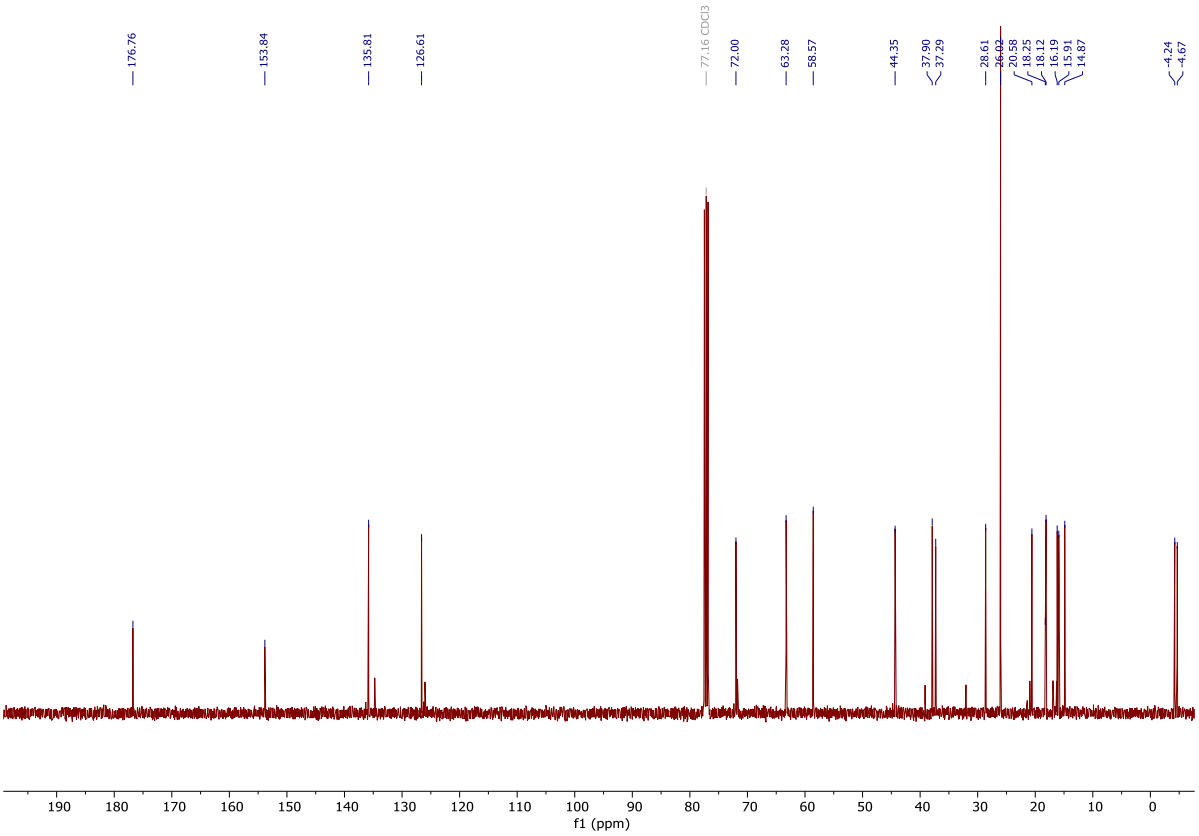
64



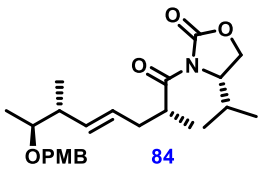
(*S*)-3-((2*R*,6*R*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-2,6-dimethyloct-4-enoyl)-4-isopropyloxazolidin-2-one 71

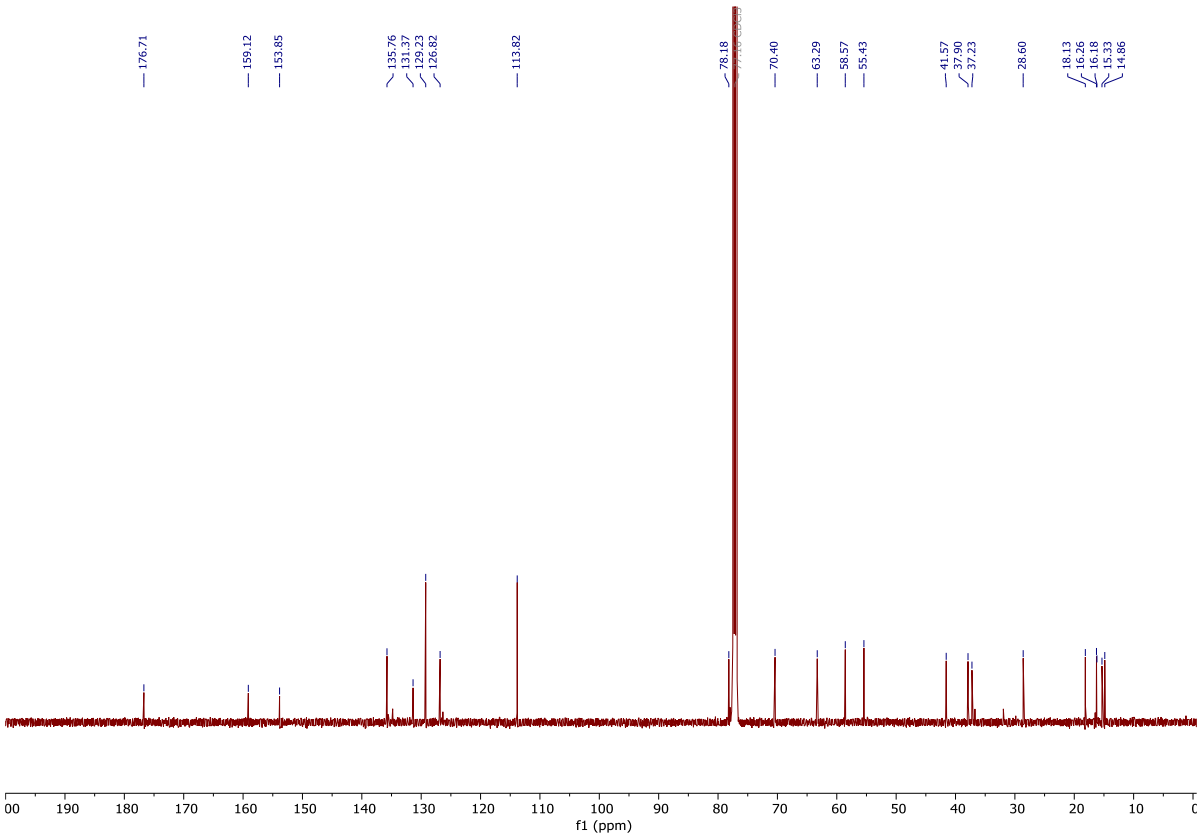


71

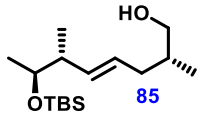


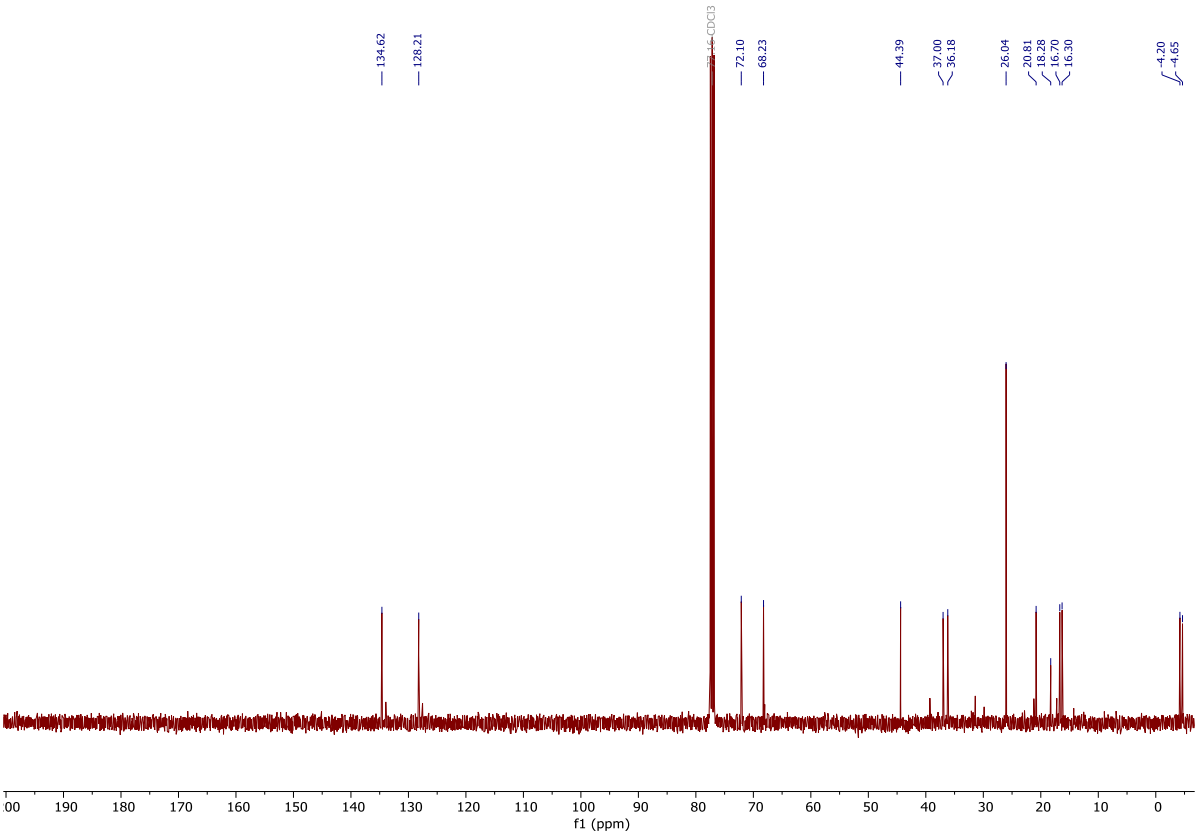
(*S*)-4-Isopropyl-3-((2*R*,6*R*,7*S*,*E*)-7-((4-methoxybenzyl)oxy)-2,6-dimethyloct-4-enoyl)oxazolidin-2-one 84



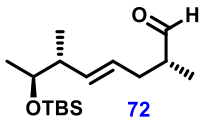


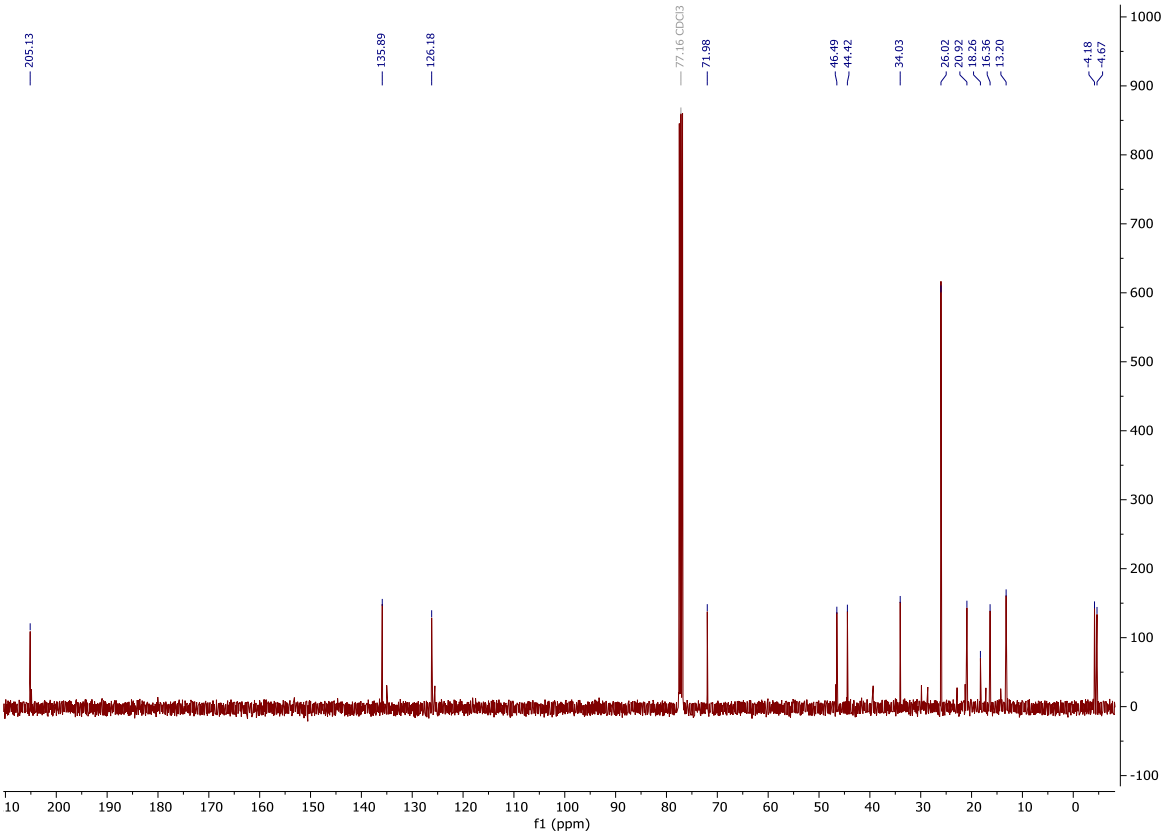
(2*R*,6*R*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-2,6-dimethyloct-4-en-1-ol 85



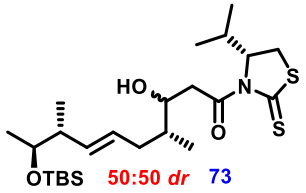


(2*R*,6*R*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-2,6-dimethyloct-4-enal 72

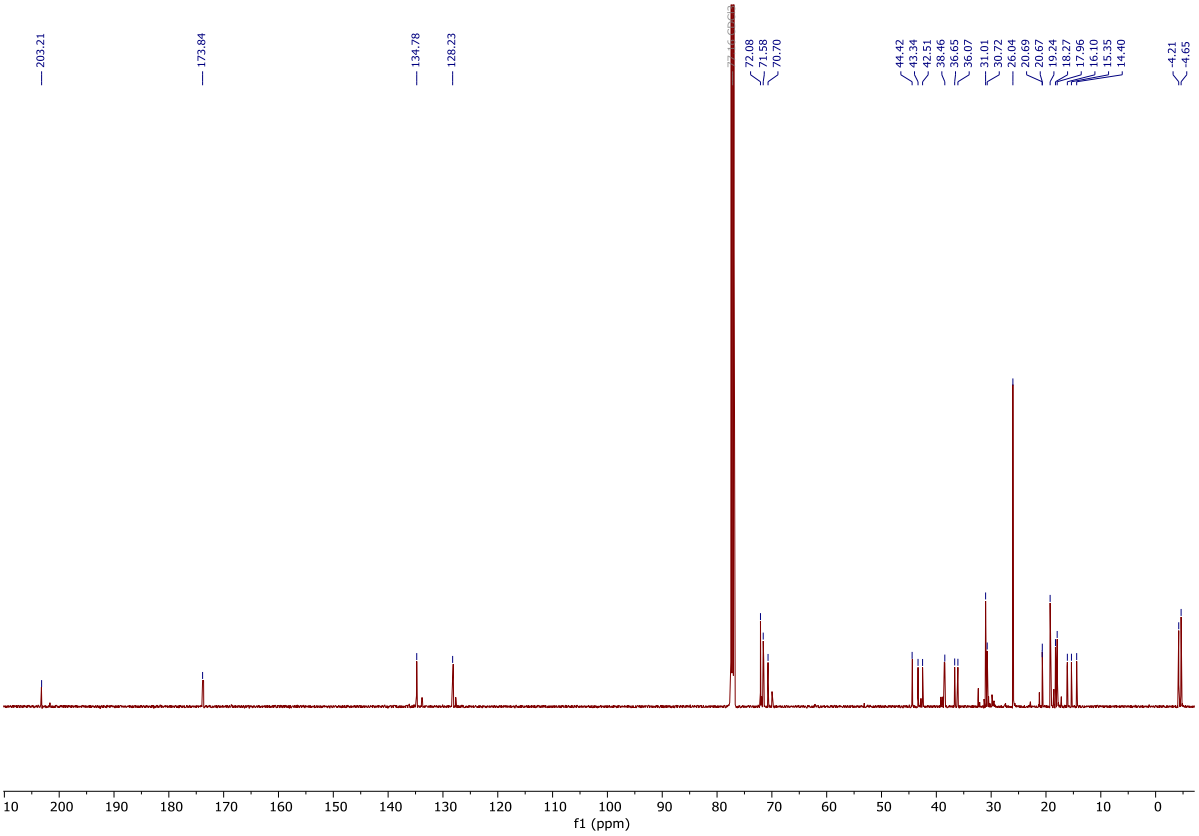




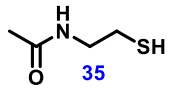
(3*S*,4*R*,8*R*,9*S*,*E*)-9-((*tert*-Butyldimethylsilyl)oxy)-3-hydroxy-1-((*R*)-4-isopropyl-2-thioxothiazolidin-3-yl)-4,8-dimethyldec-6-en-1-one 73

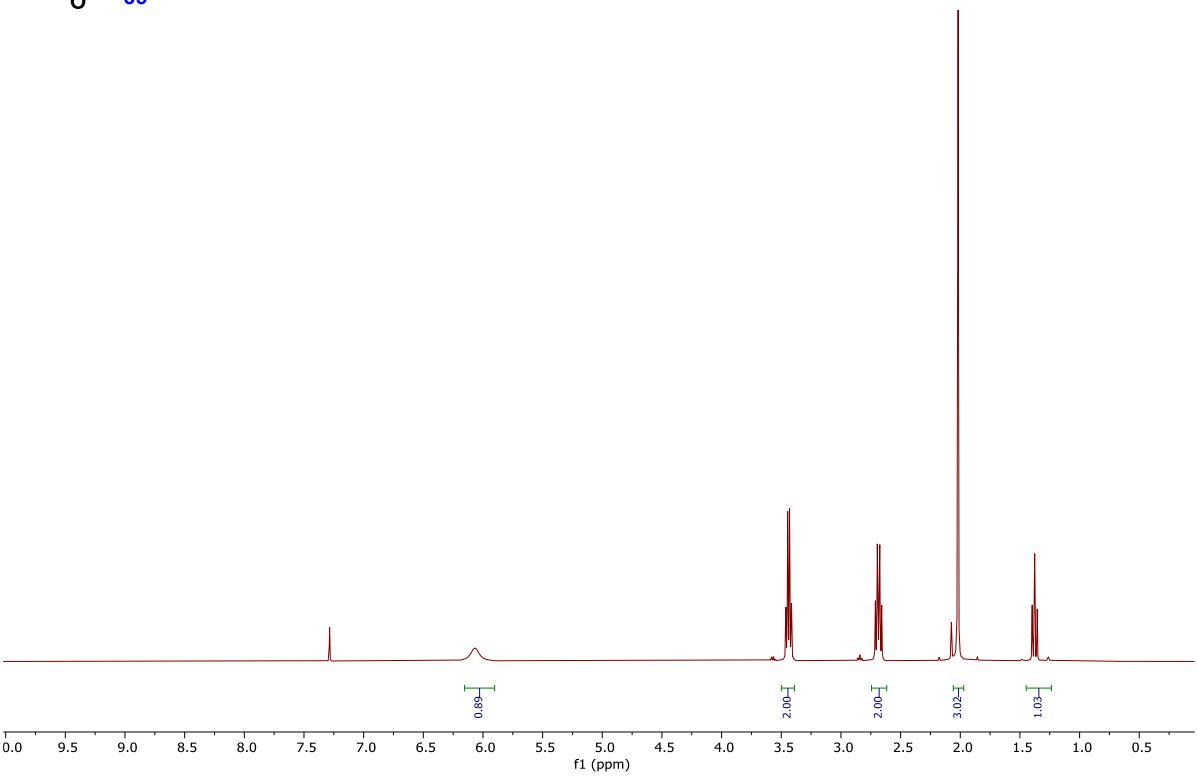


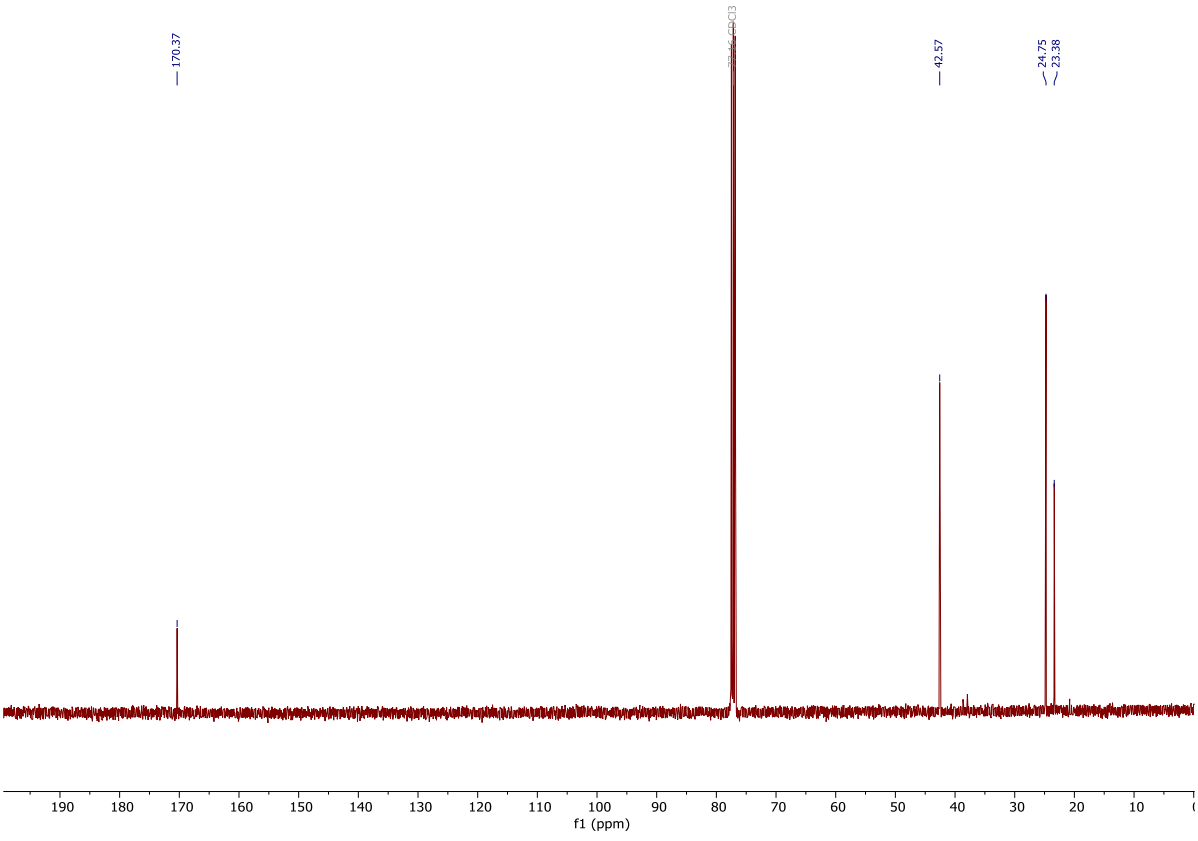
50:50 *dr* 73



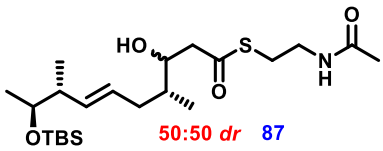
N-acetylcysteamine 35

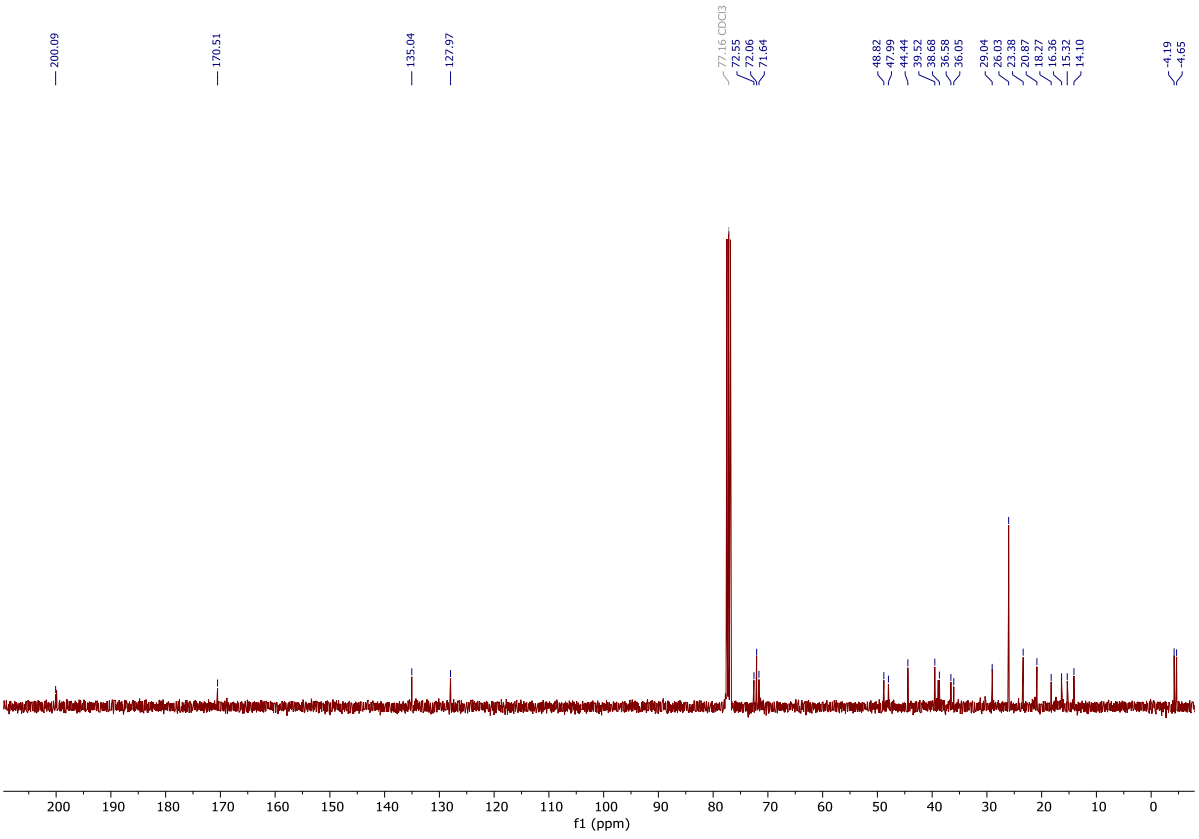




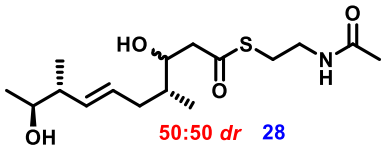


_S_-(2-acetamidoethyl)(4_R_,8_R_,9_S_,_E_)-9-((_tert_-butyldimethylsilyl)oxy)-3-hydroxy-4,8-dimethyldec-6-enethioate 87

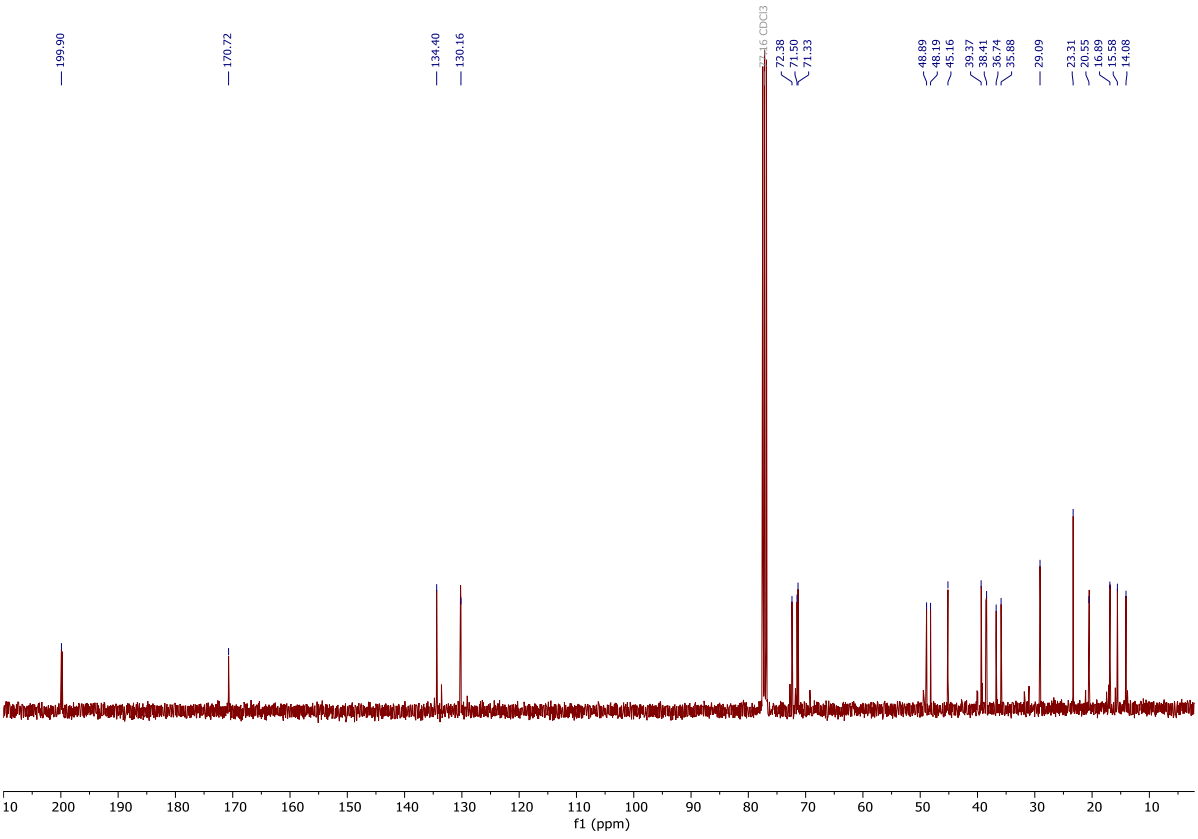




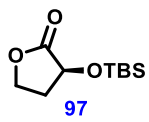
_S_-(2-acetamidoethyl) (4_R_,8_R_,9_S_,_E_)-3,9-dihydroxy-4,8-dimethyldec-6-enethioate 28



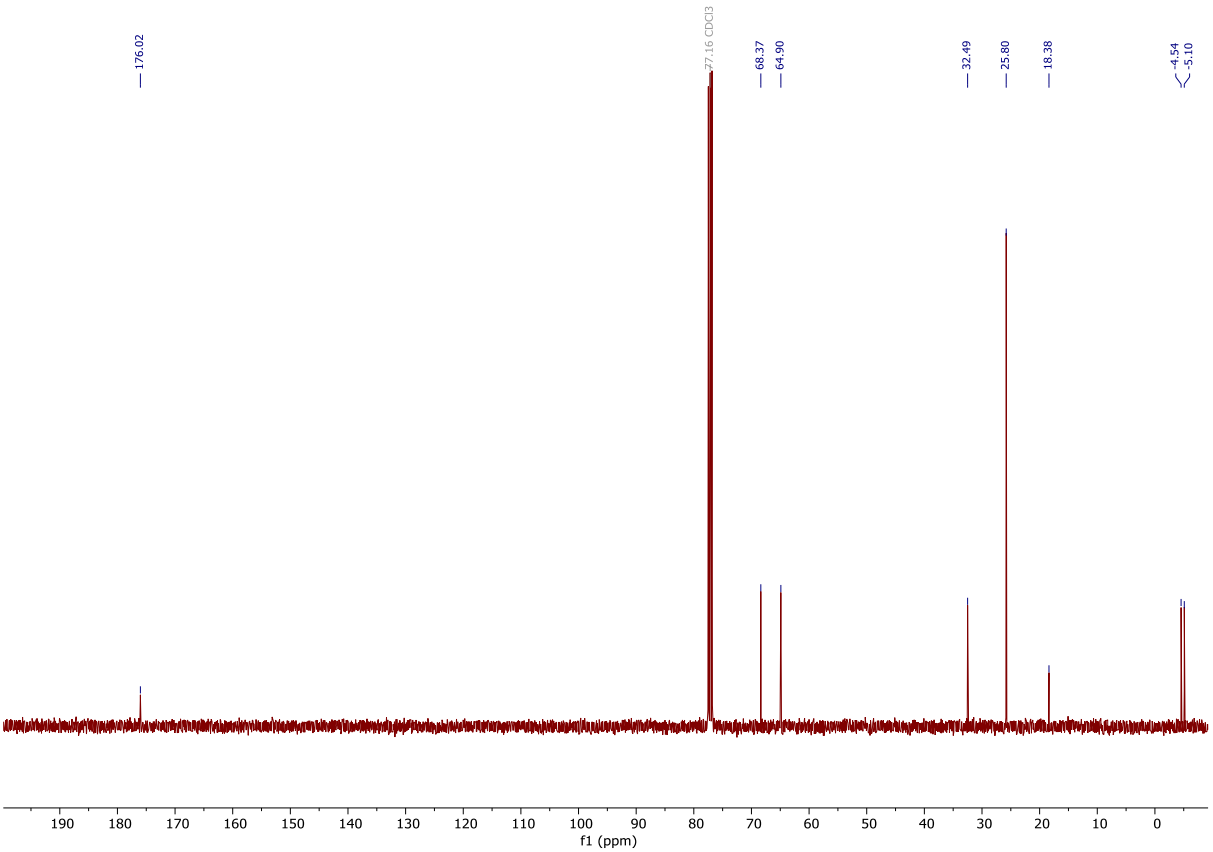
50:50 *dr* 28



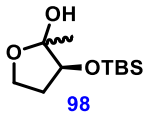
(*S*)-3-(*tert*-Butyldimethylsilyloxy)dihydrofuran-2(3H)-one 97



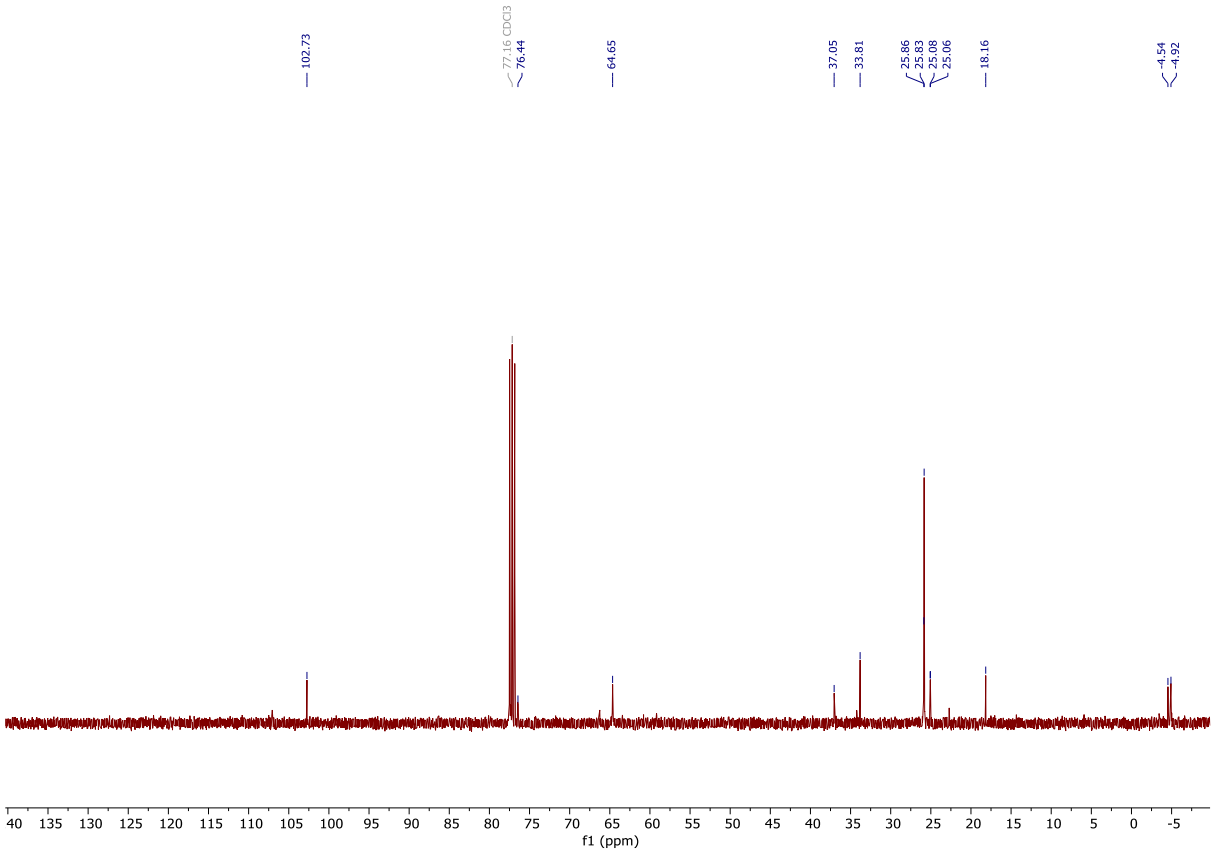
97



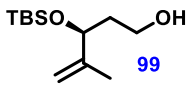
(3*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-methyltetrahydrofuran-2-ol 98

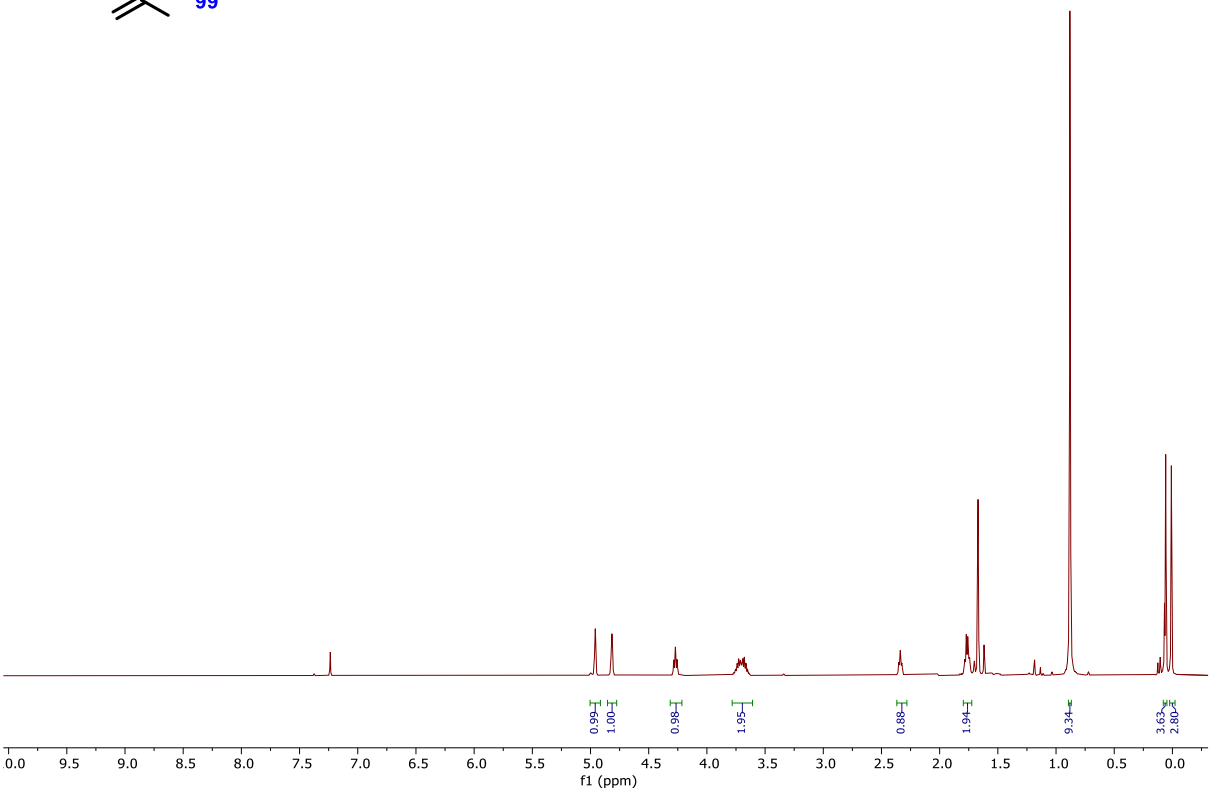


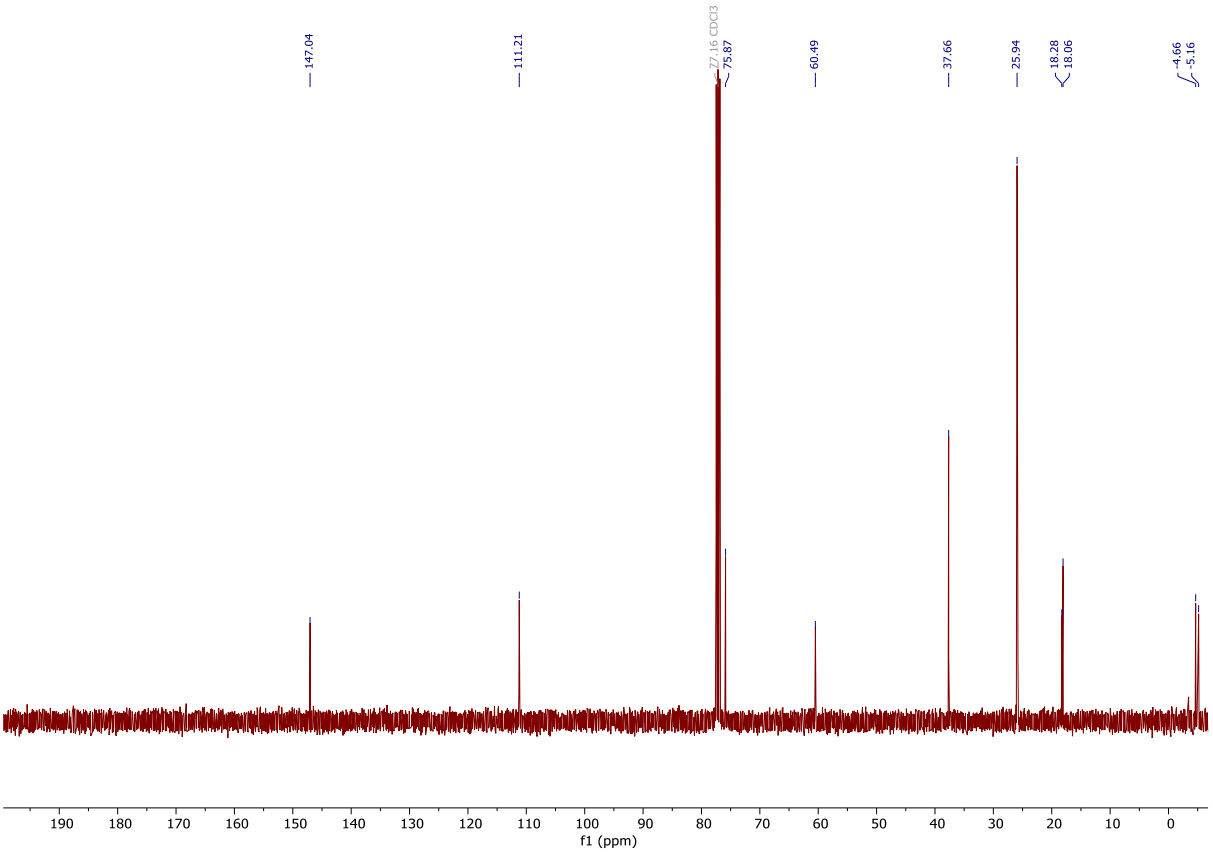
98



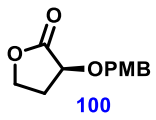
(*S*)-3-(*tert*-Butyldimethylsilyloxy)-4-methylpent-4-en-1-ol 99



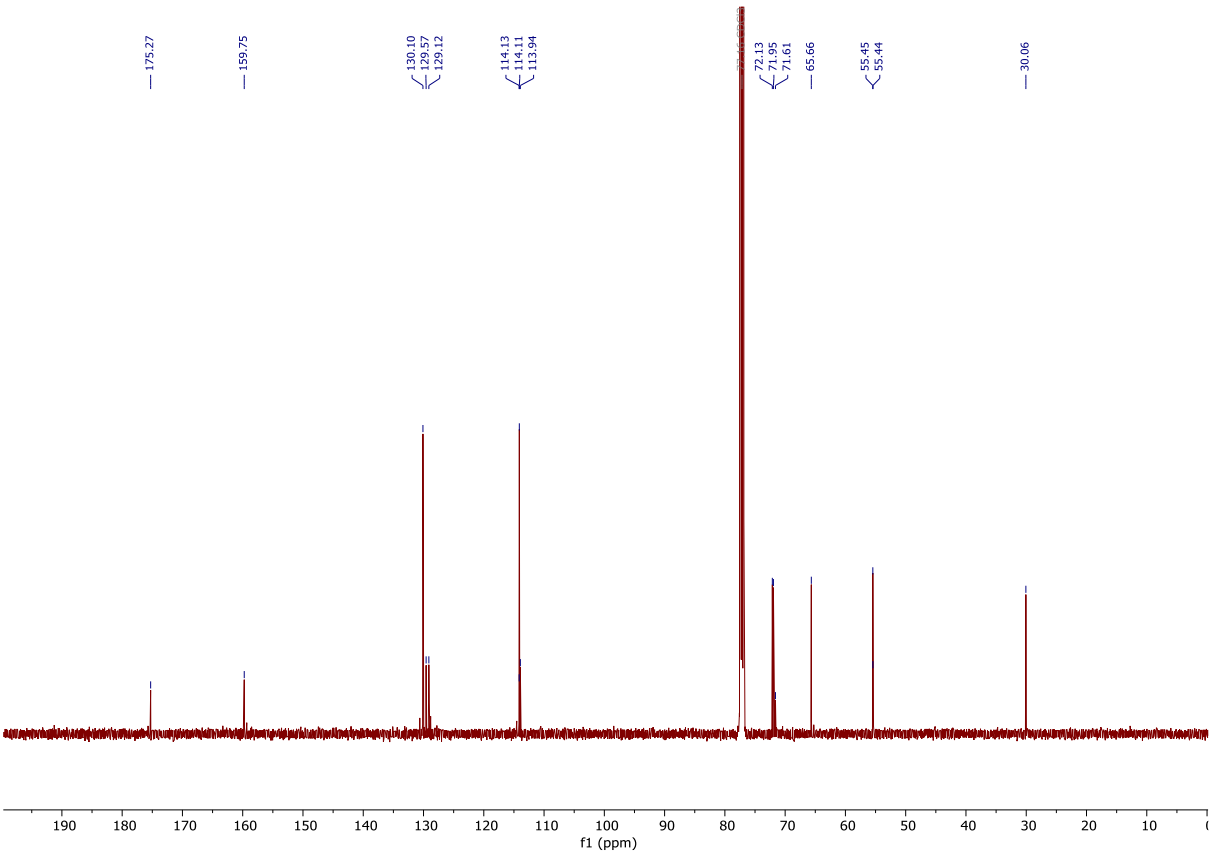




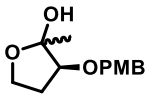
(*S*)-3-((4-Methoxybenzyl)oxy)dihydrofuran-2(3H)-one 100



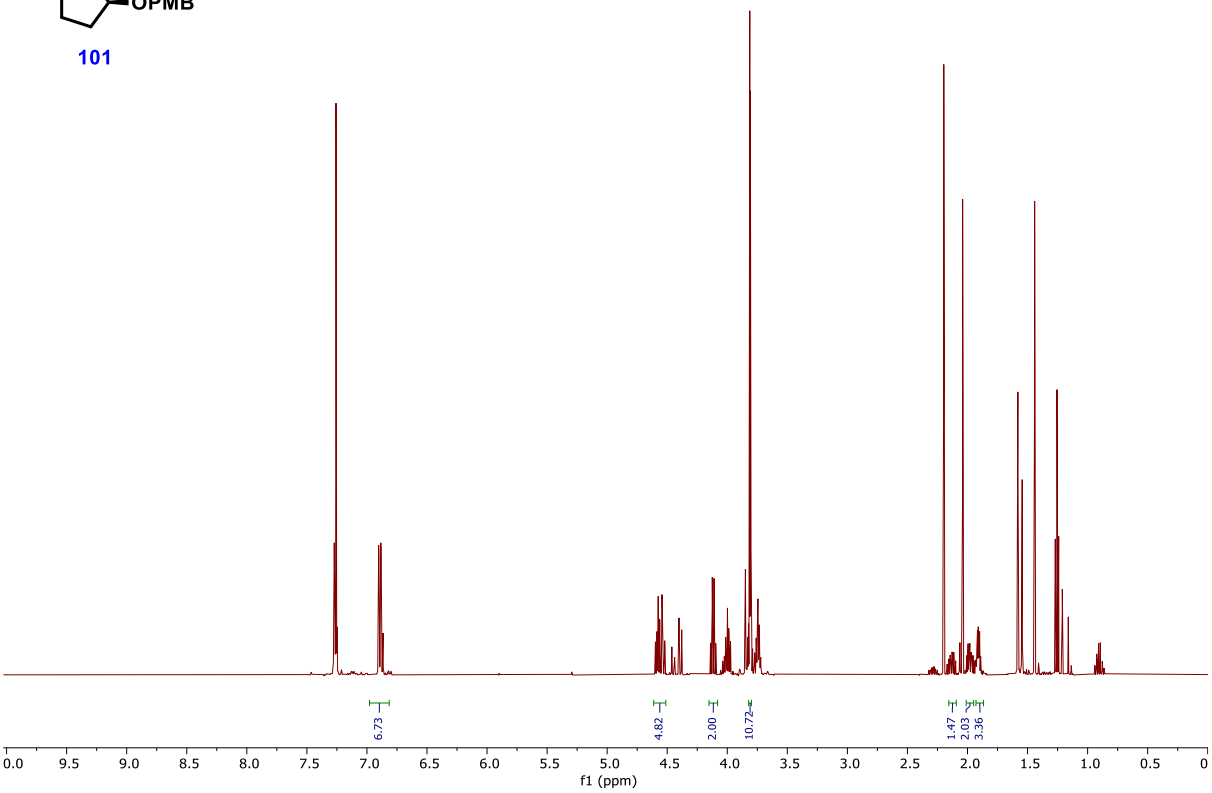
100

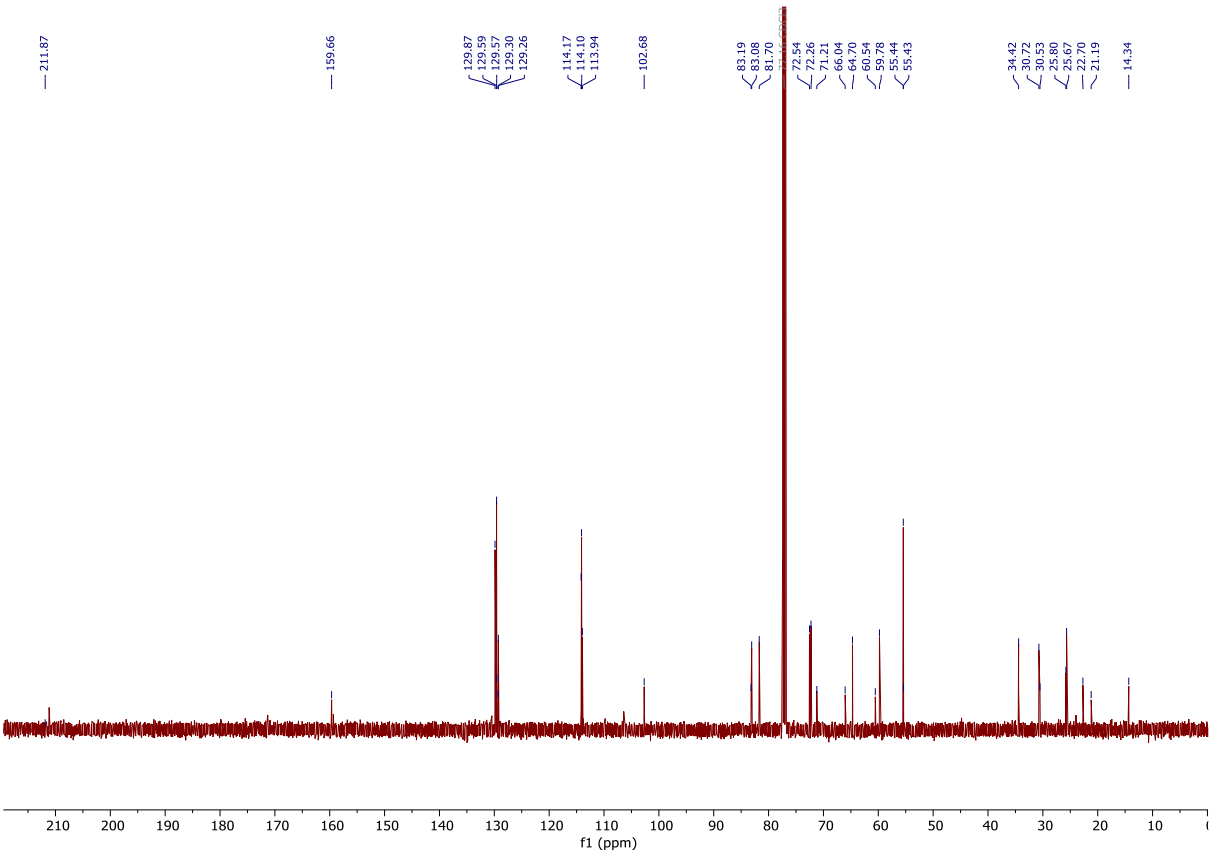


(3*S*)-3-(4-Methoxybenzyloxy)-2-methyltetrahydrofuran-2-ol 101

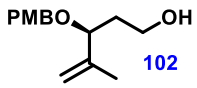


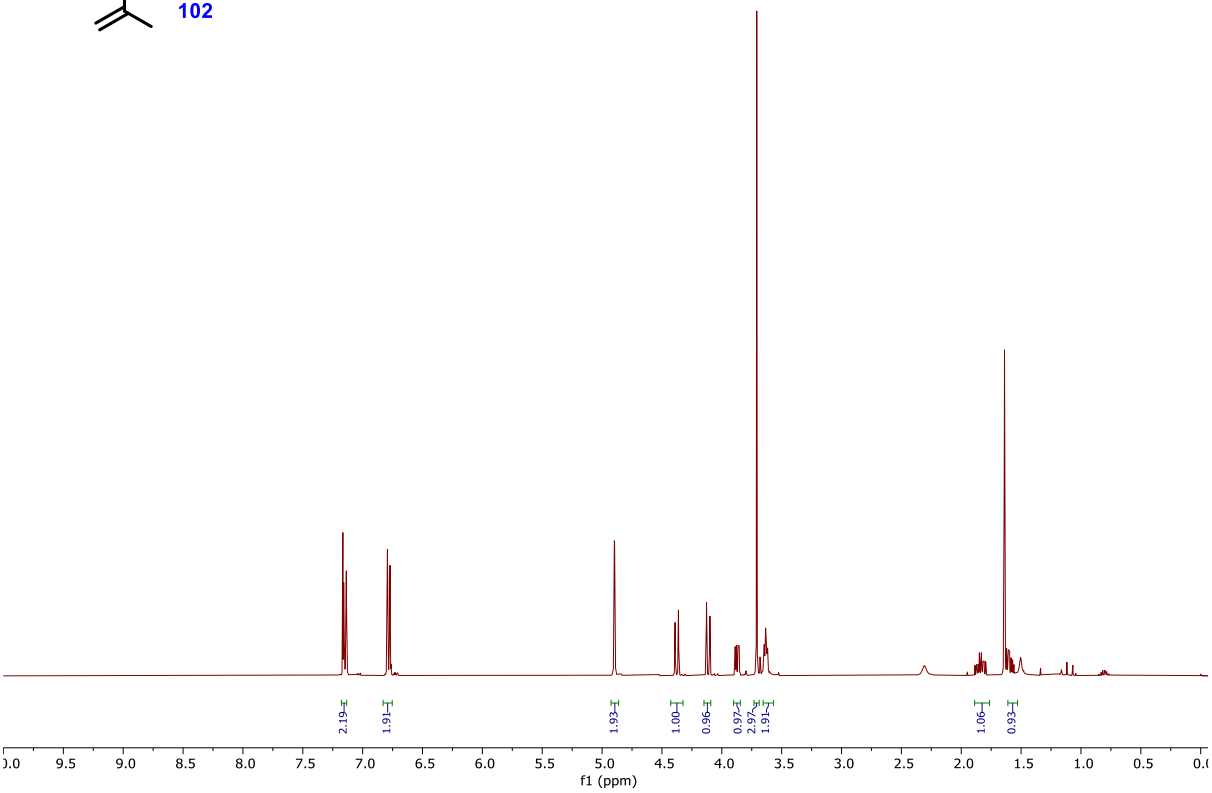
101

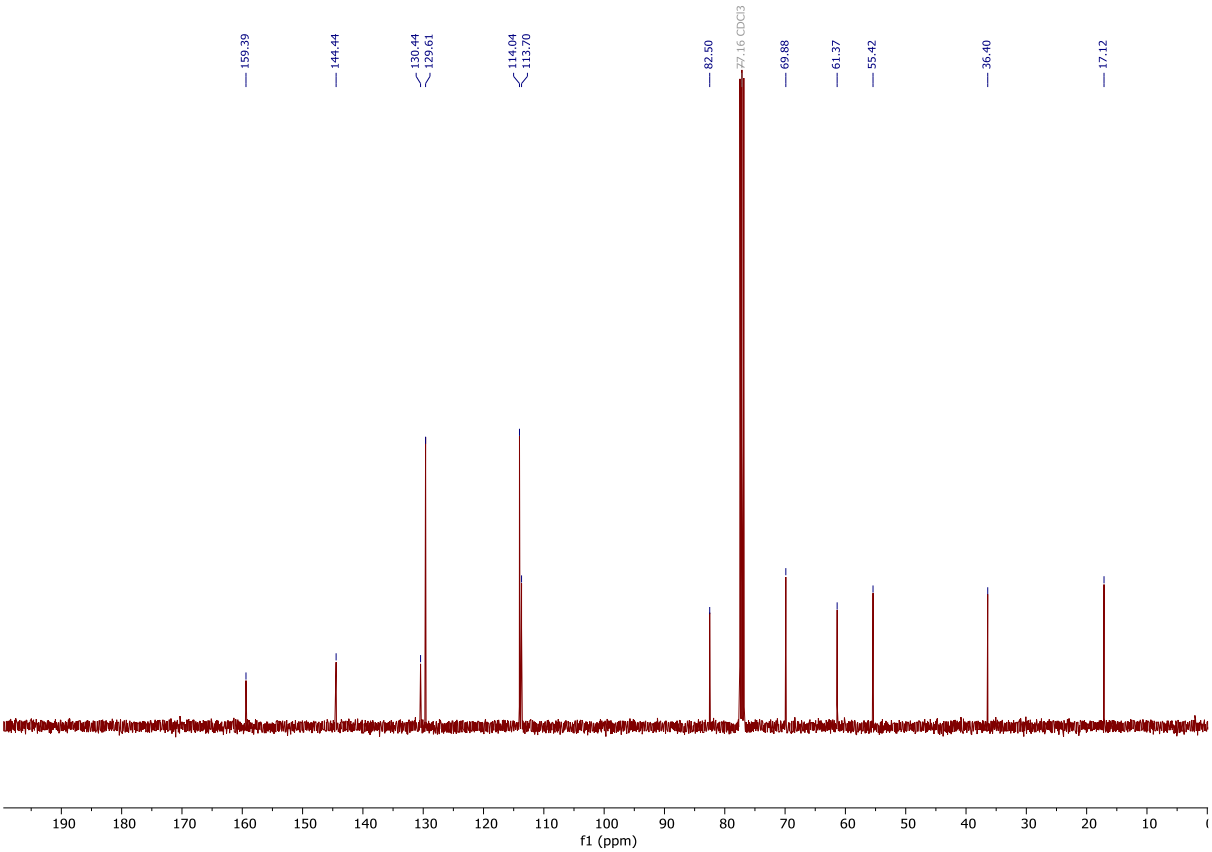




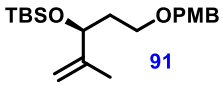
(*S*)-3-(4-Methoxybenzyloxy)-4-methylpent-4-en-1-ol 102

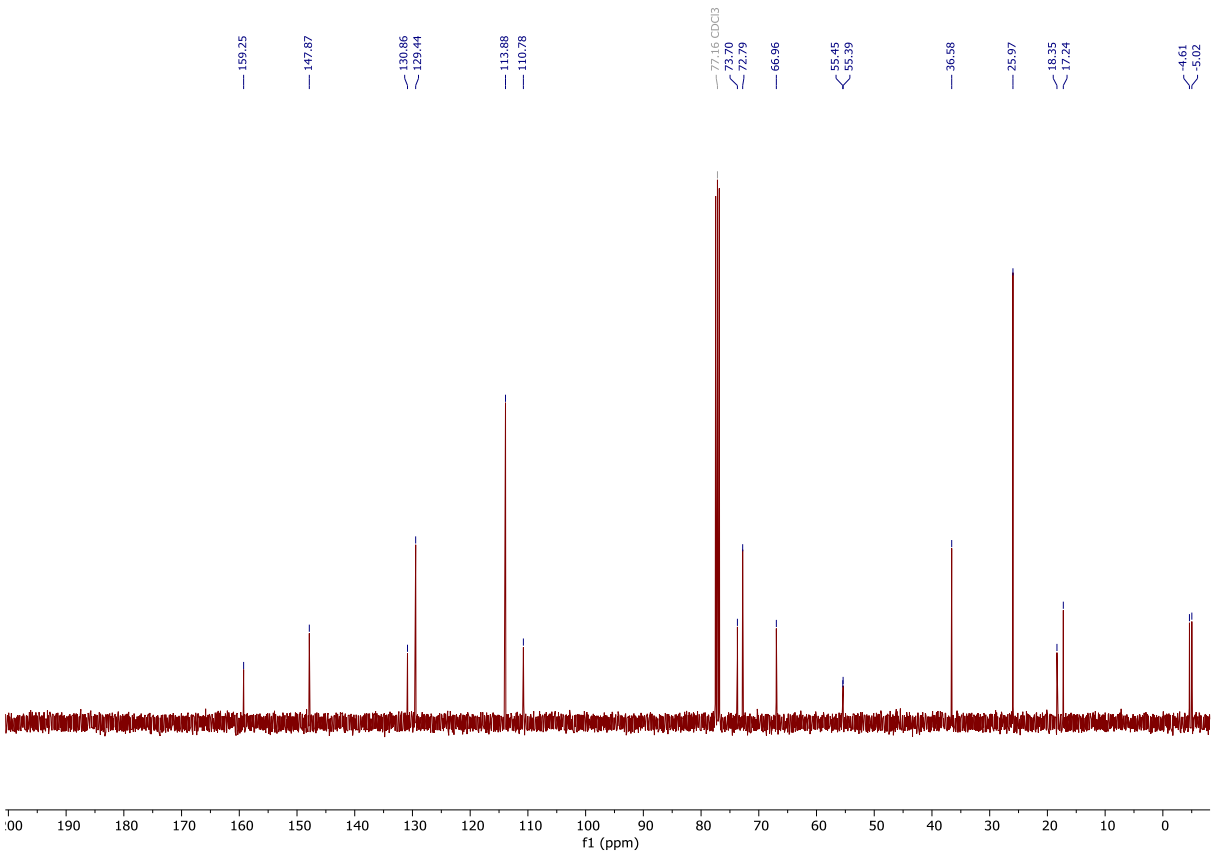




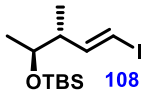


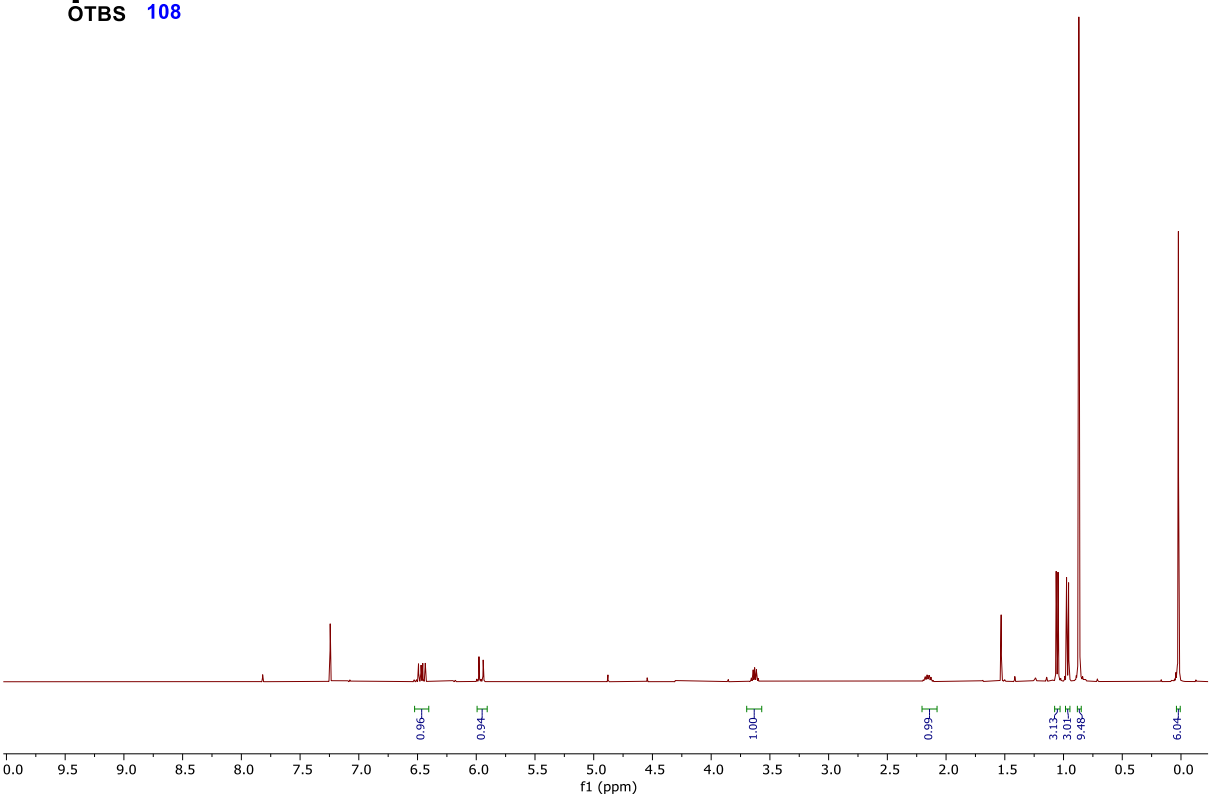
5-(*para*-Methoxybenzyloxy)-(*S*)-3-(*tert*-butyldimethylsilyloxy)-2-methylpent-1-ene 91

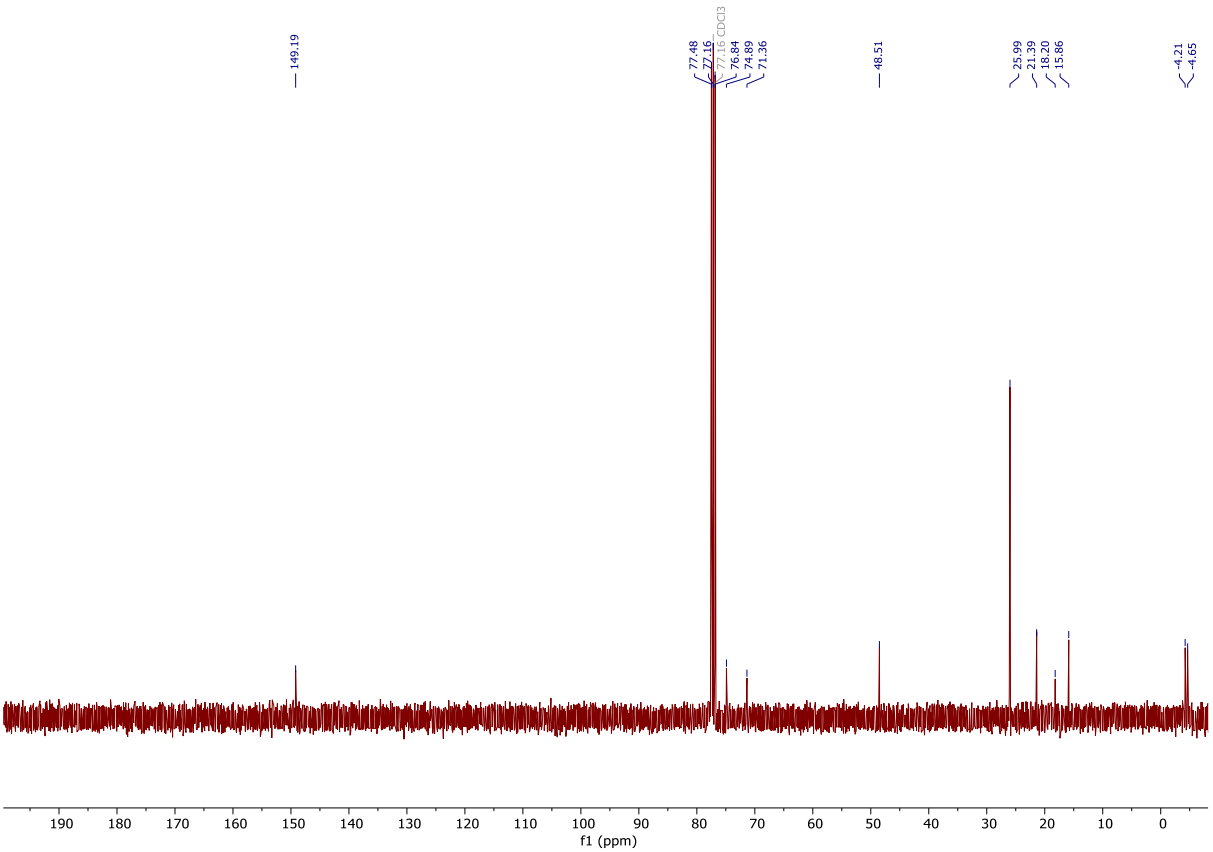




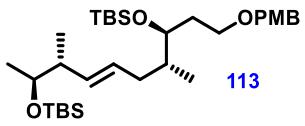
4*S*-(*tert*-butyldimethylsilyloxy)-3*R*,*E*-1-iodo-3-methylpent-1-ene 108



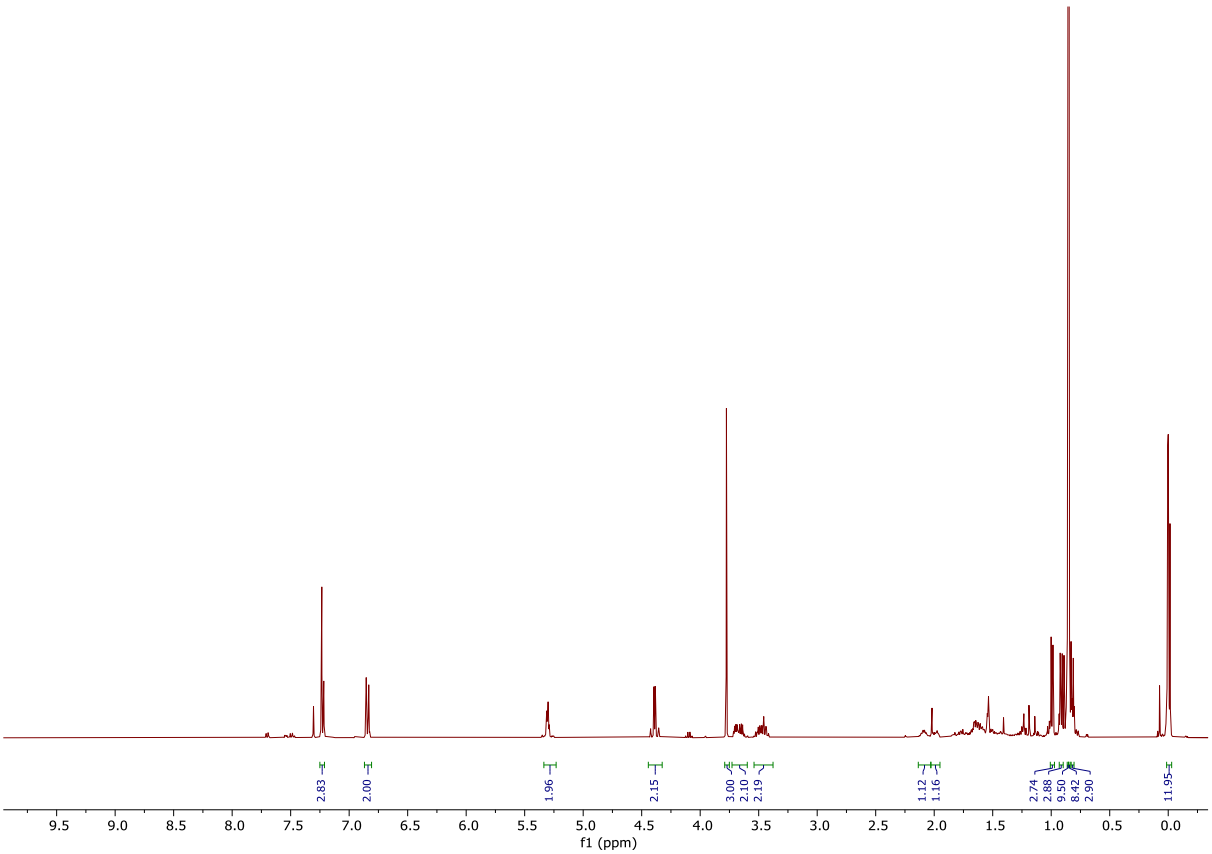




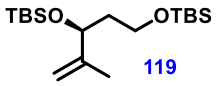
(3*S*,4*R*,8*R*,9*S*,*E*)-1-(*para*-Methoxybenzyloxy)-3,9-bis(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-ene 113



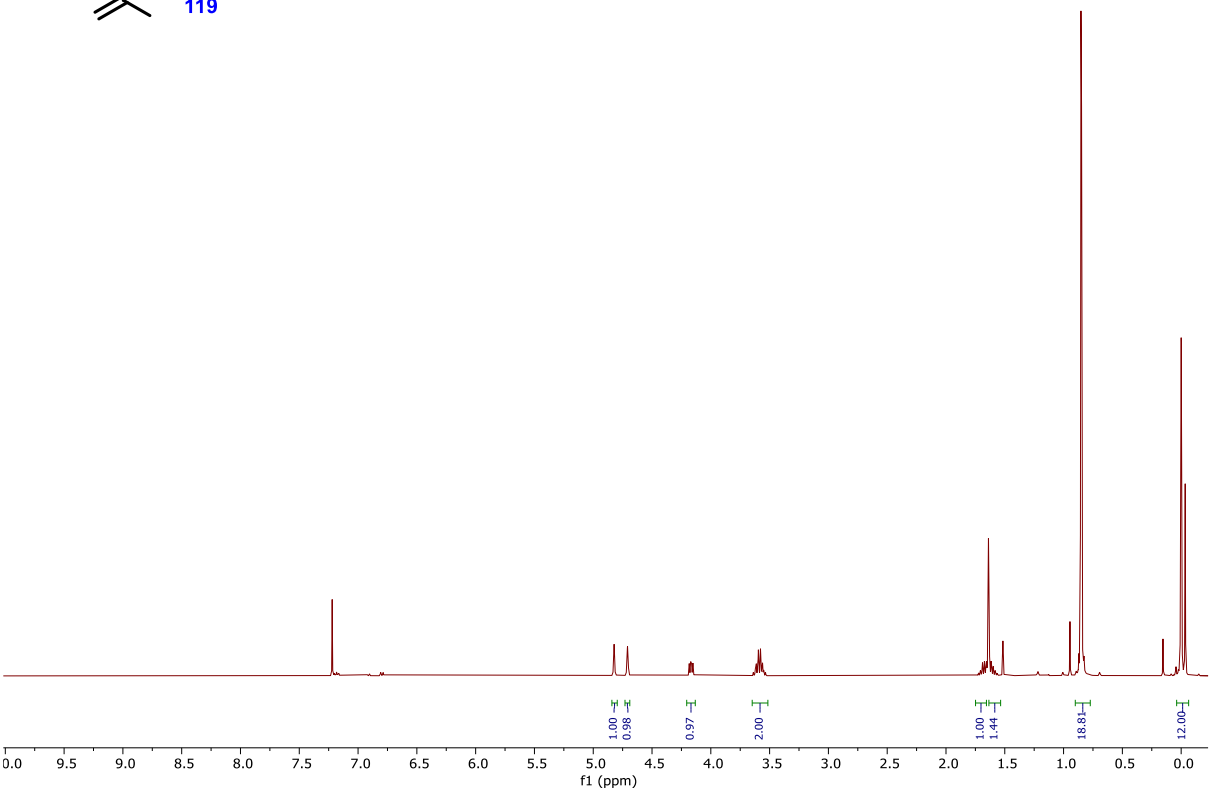
113

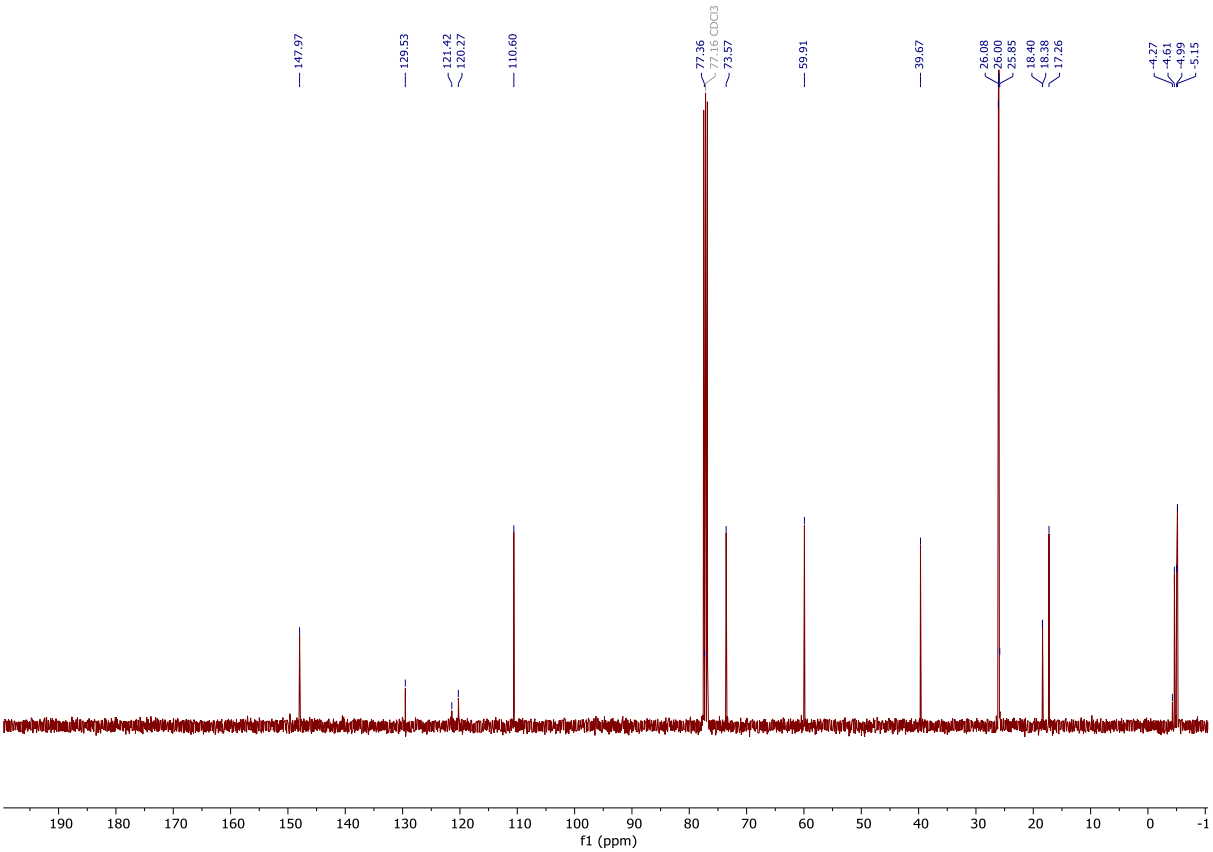


(*S*)-1,3-Bis(*tert*-butyldimethylsilyloxy)-4-methylpent-4-ene 119

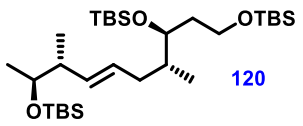


119

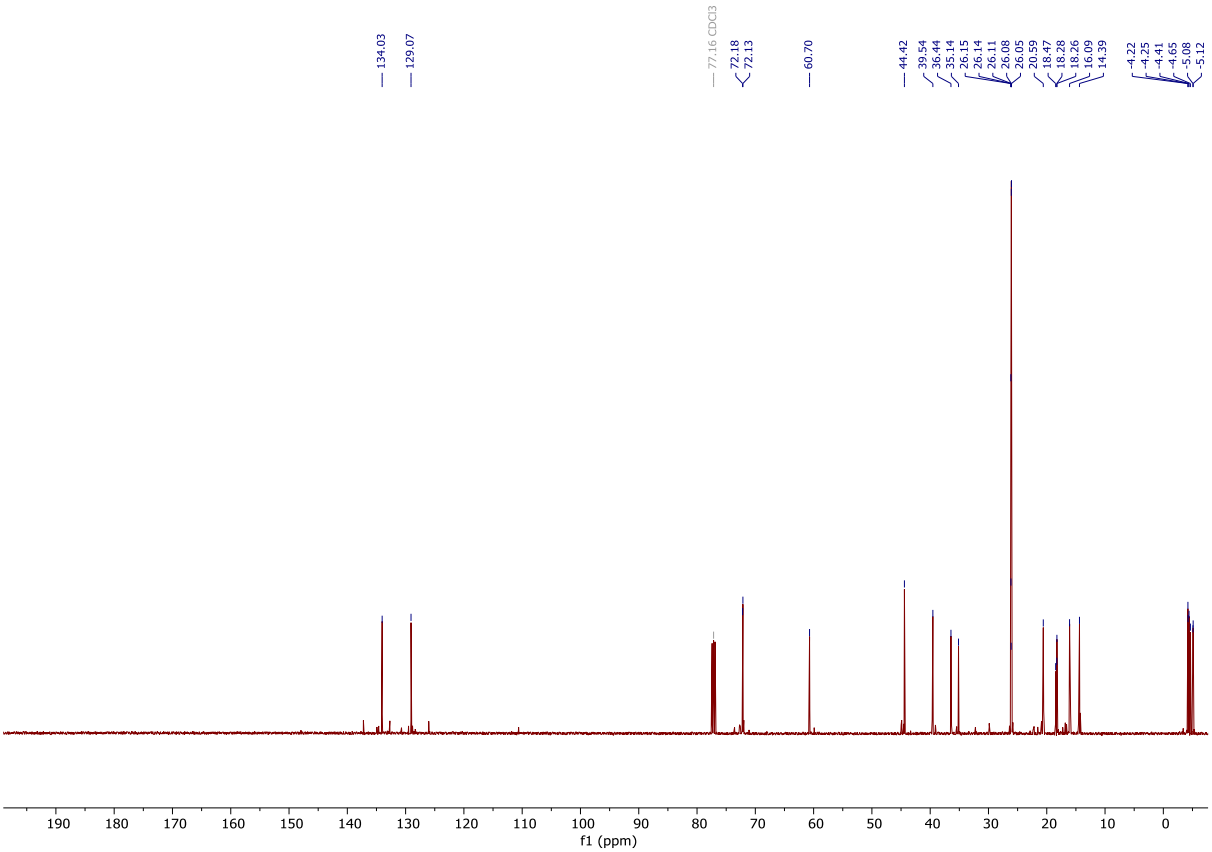




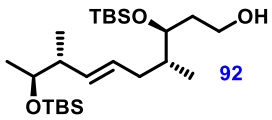
(3*S*,4*R*,8*R*,9*S*,*E*)-1,3,9-Tris(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-ene 120

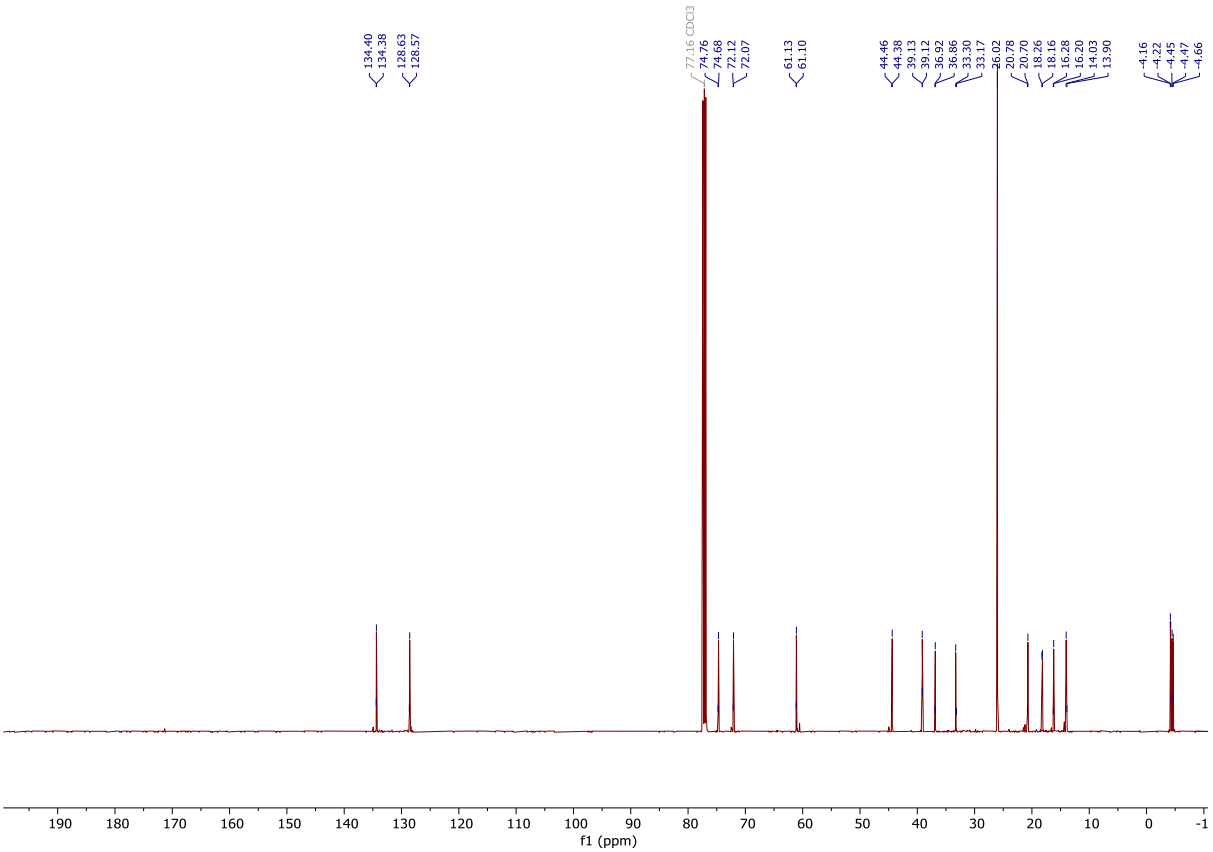


120

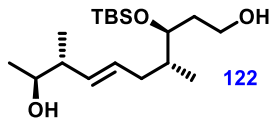


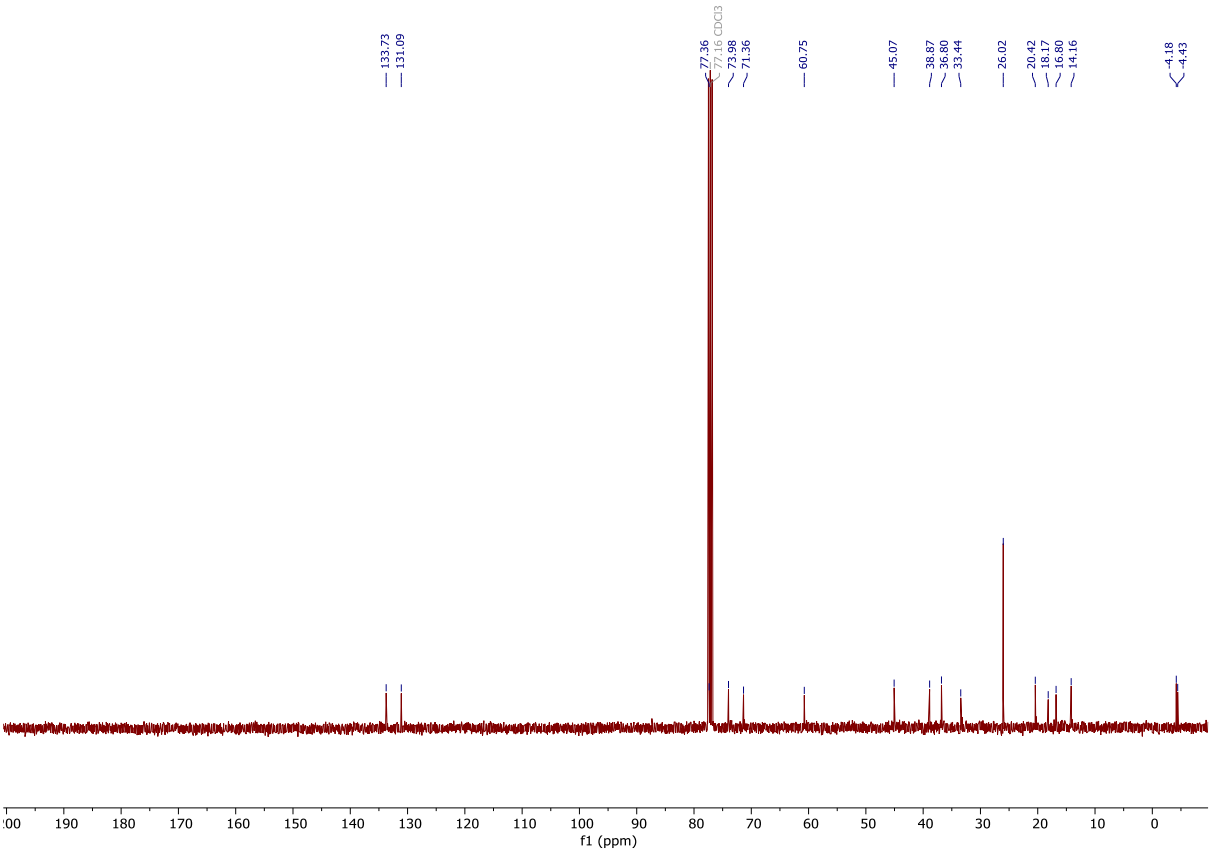
(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-Bis(*tert*-butyldimethylsilyloxy)-4,8-dimethyldec-6-en-1-ol 92



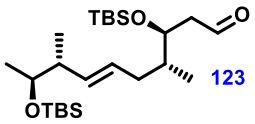


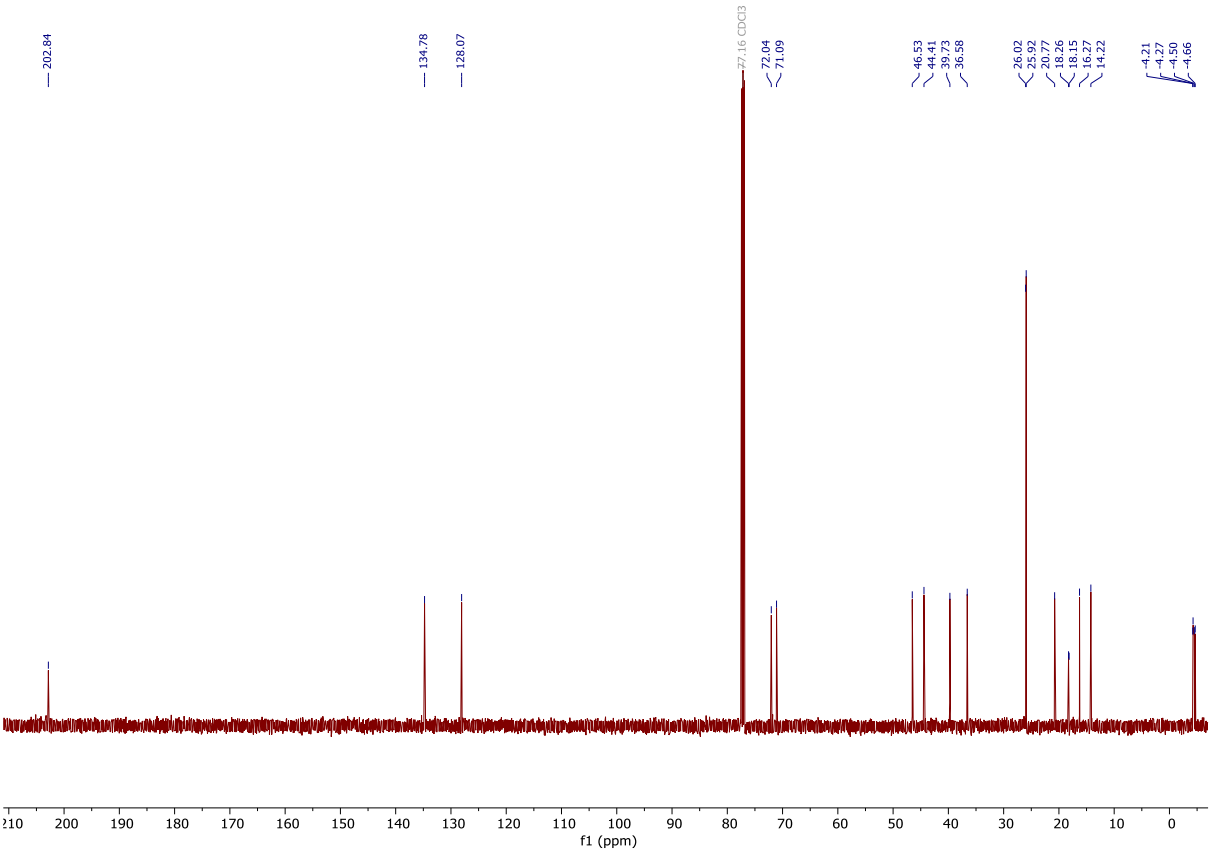
(3*S*,4*R*,8*R*,9*S*,*E*)-3-(*tert*-butyldimethylsilyloxy)-9-hydroxy-4,8-dimethyldec-6-en-1-ol 122



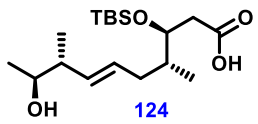


(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-bis(*tert*-Butyldimethylsilyloxy)-4,8-dimethyldec-6-enal 123

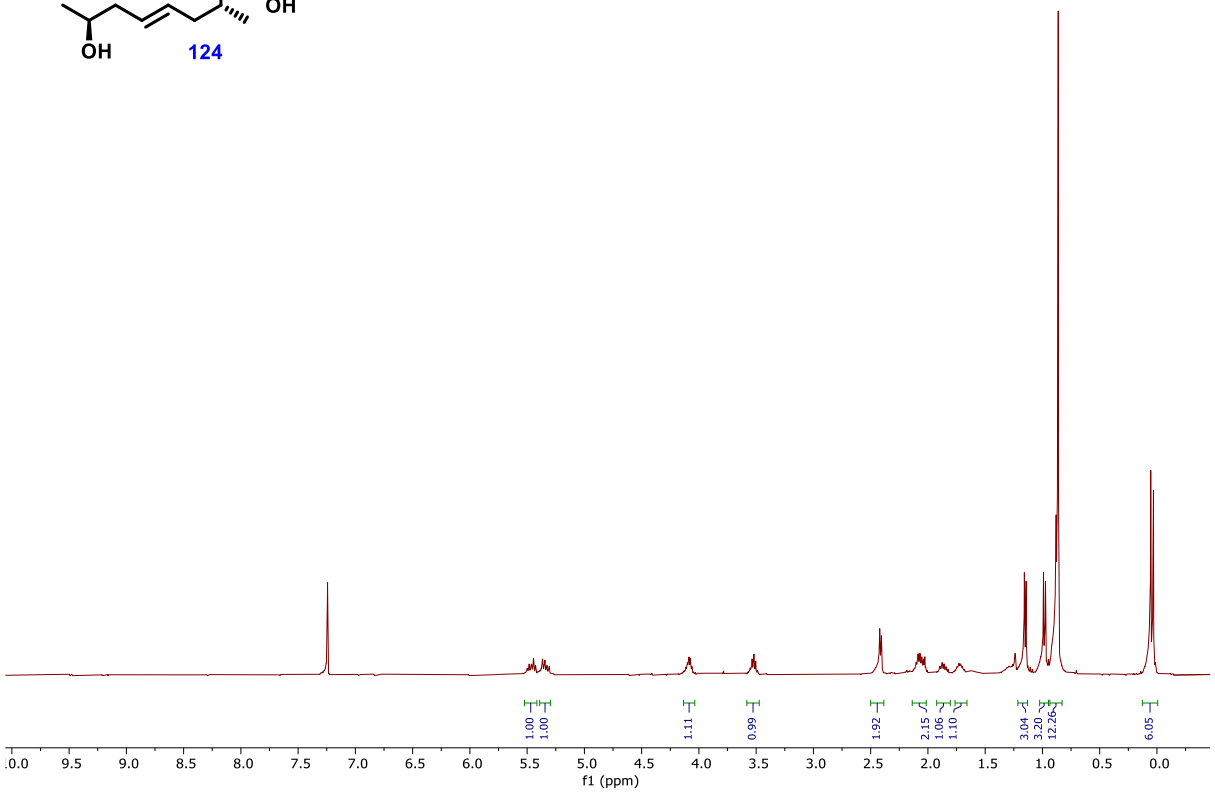


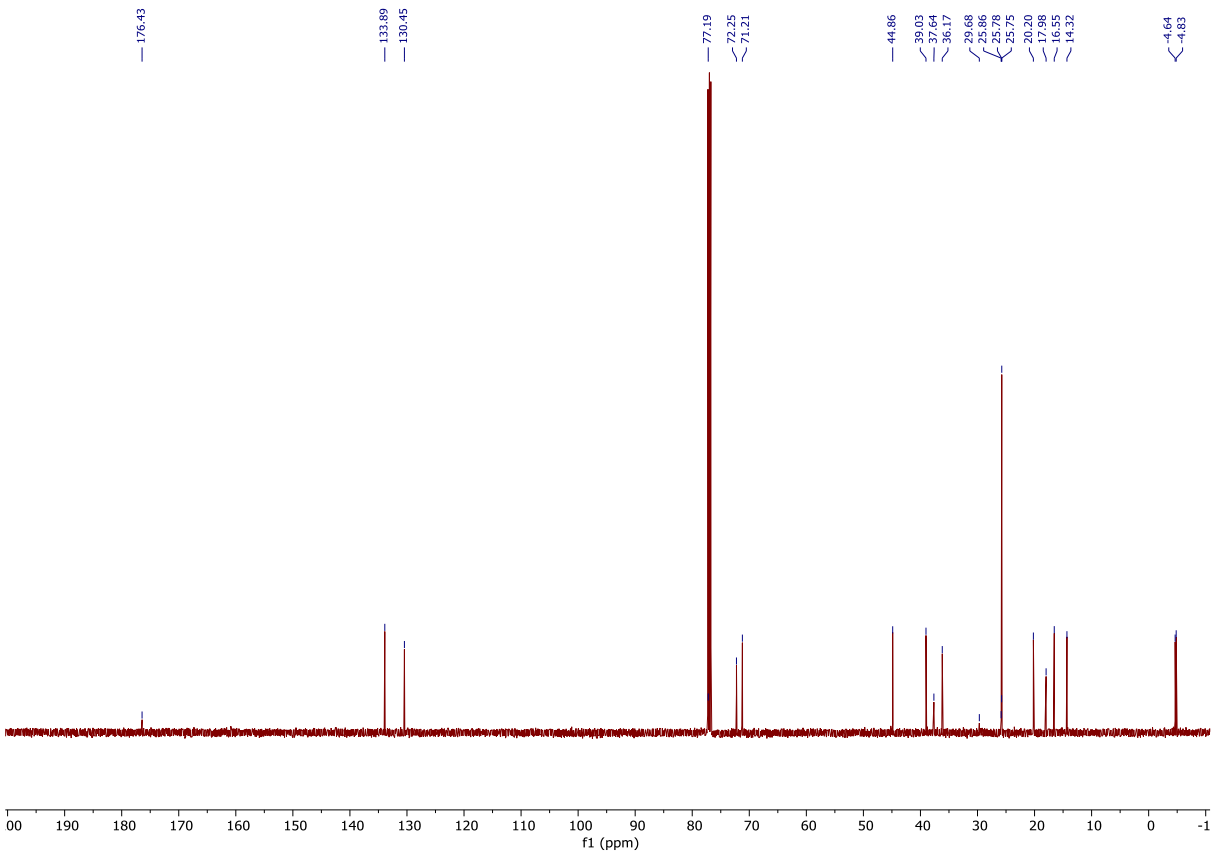


(3*S*,4*R*,8*R*,9*S*,*E*)-3-(*tert*-Butyldimethylsilyloxy)-9-hydroxy-4,8-dimethyldec-6-enoic acid 124

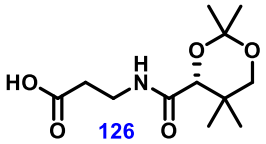


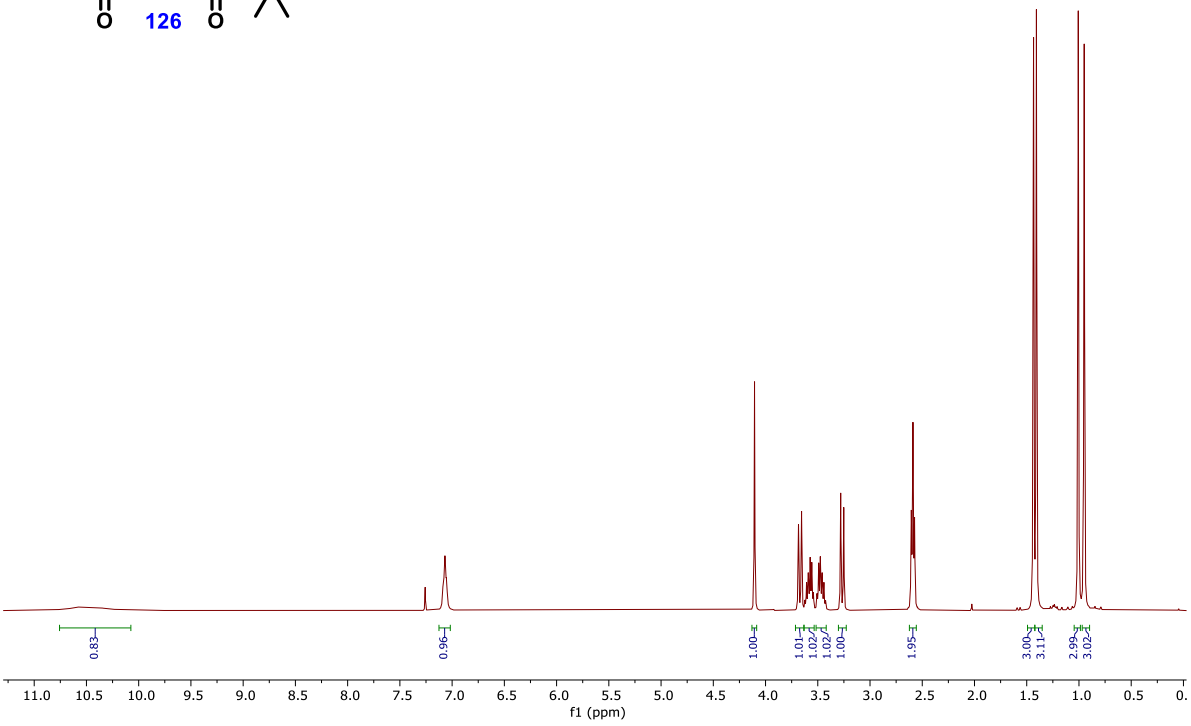
124

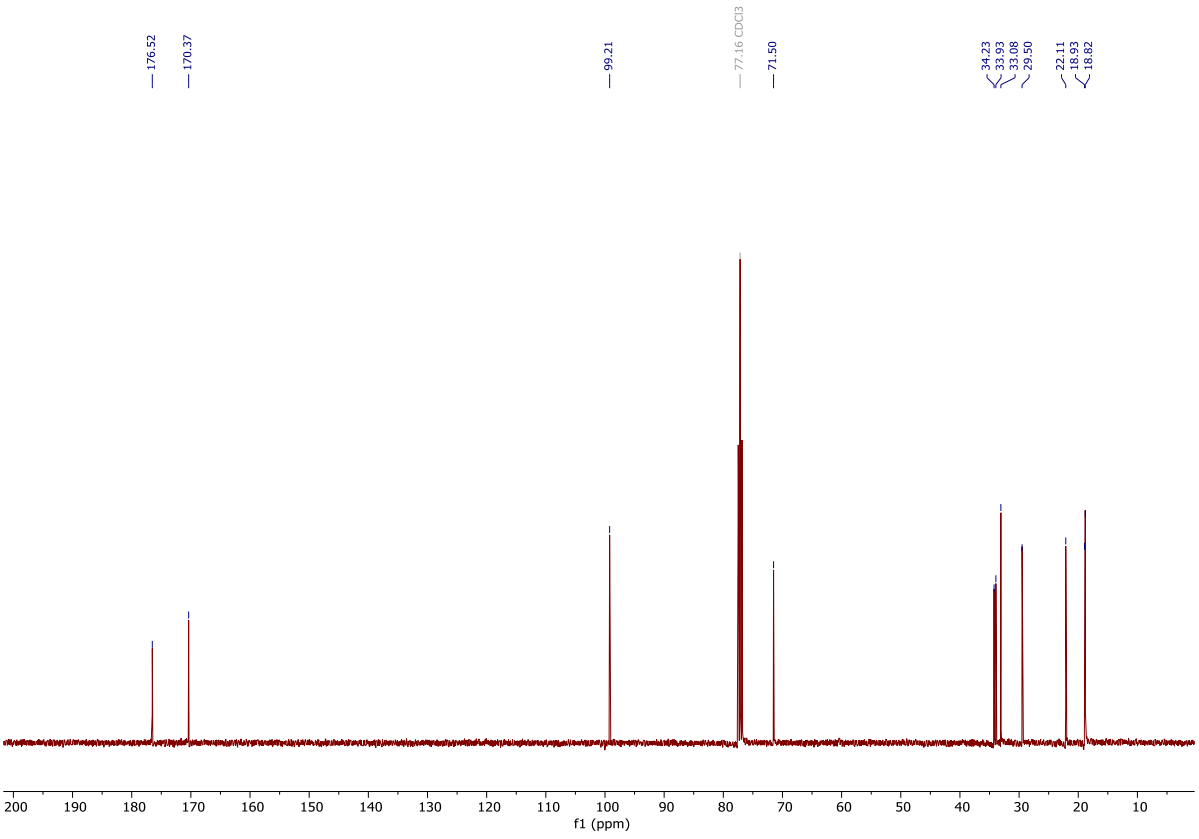




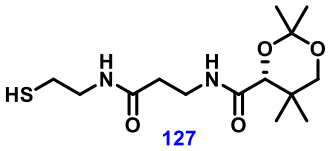
(*R*)-3-(2,2,5,5-tetramethyl-1,3-dioxane-4-carboxamido)propionic acid 126

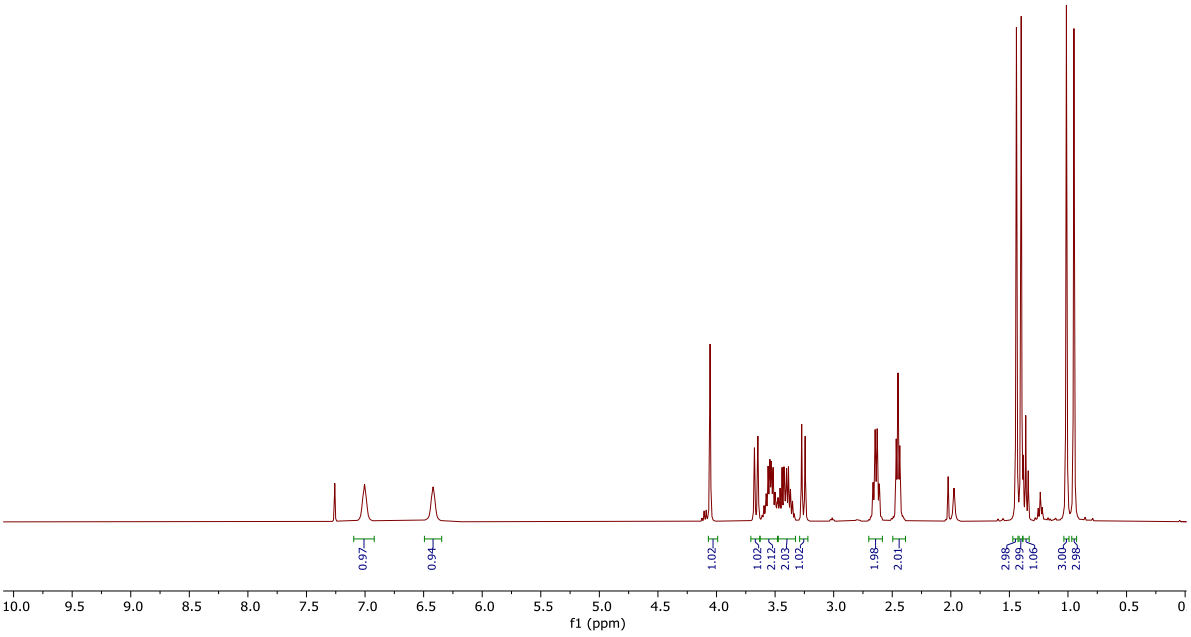


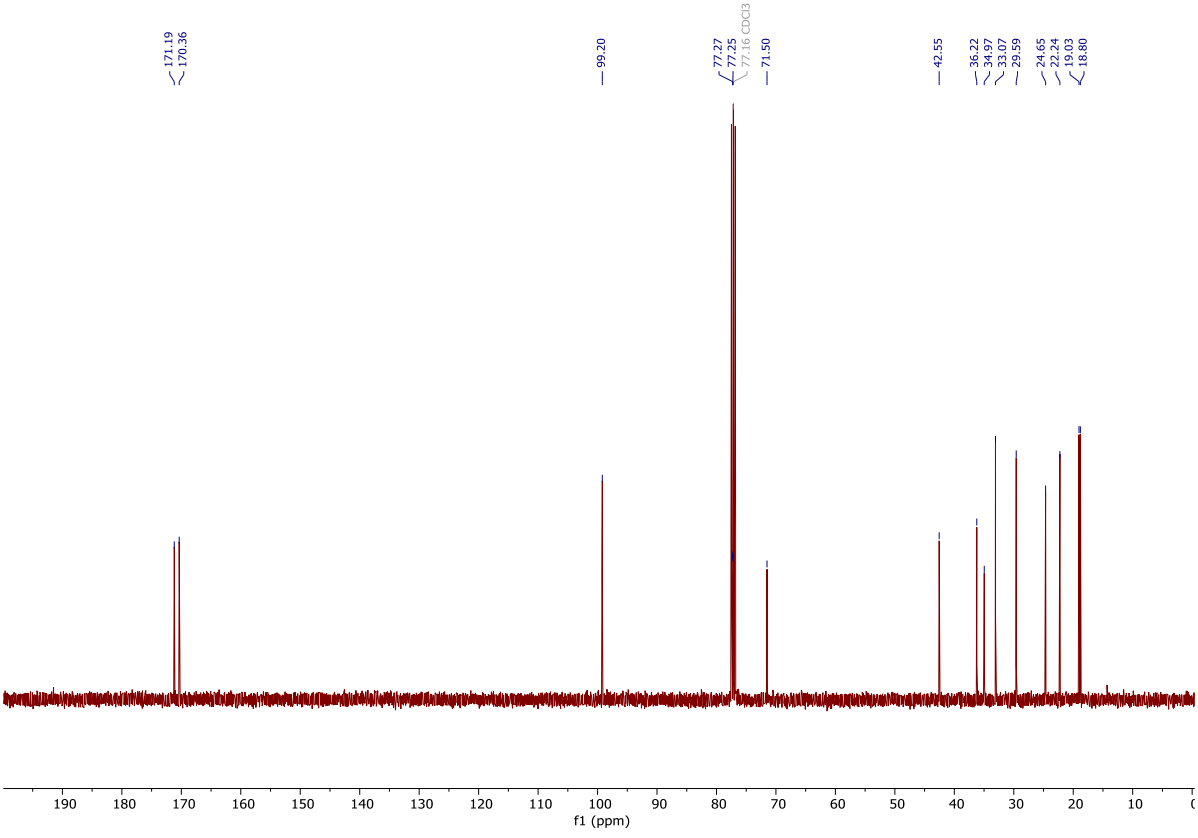




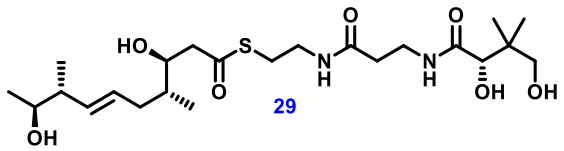
Pantetheine dimethyl ketal 127

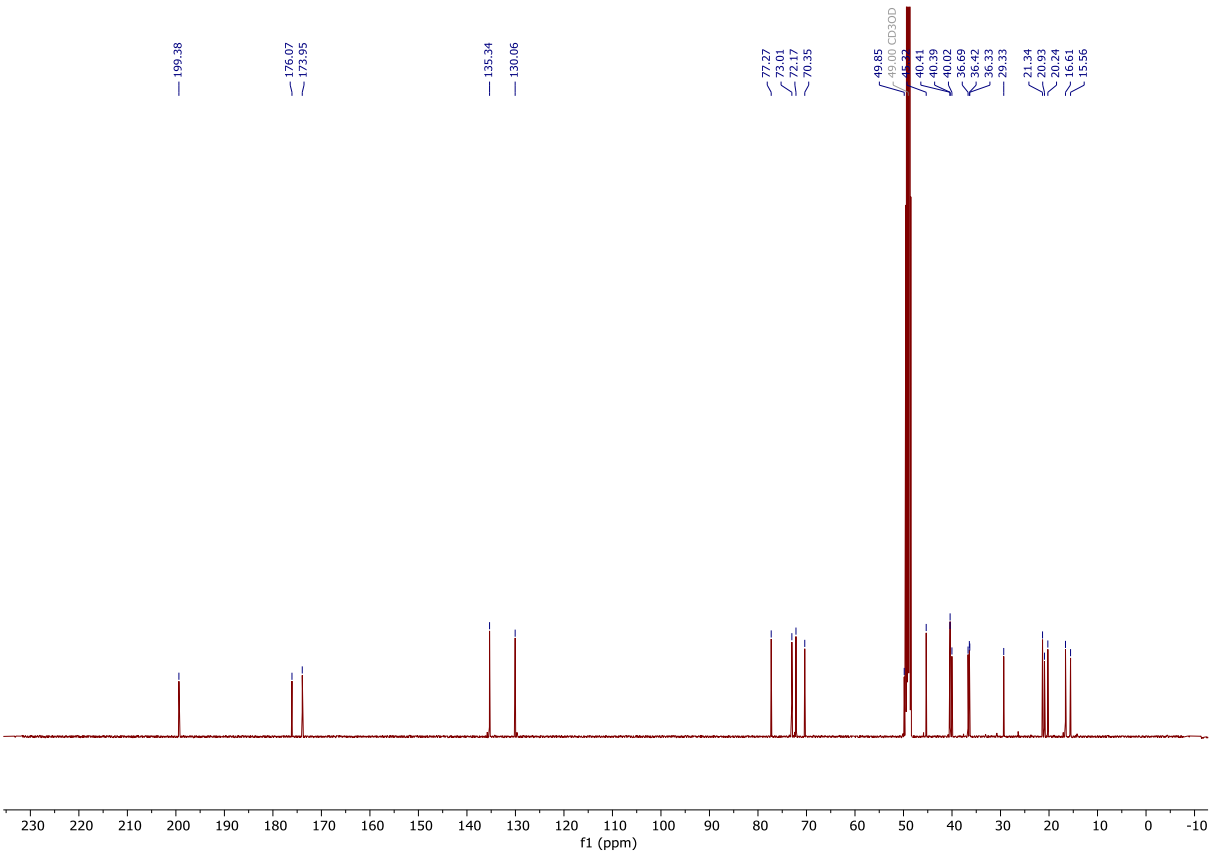




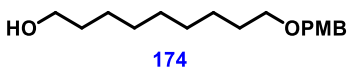


(3*S*,4*R*,8*R*,9*S*,*E*)-3,9-dihydroxy-4,8-dimethyldec-6-enethioate-pantetheine 29

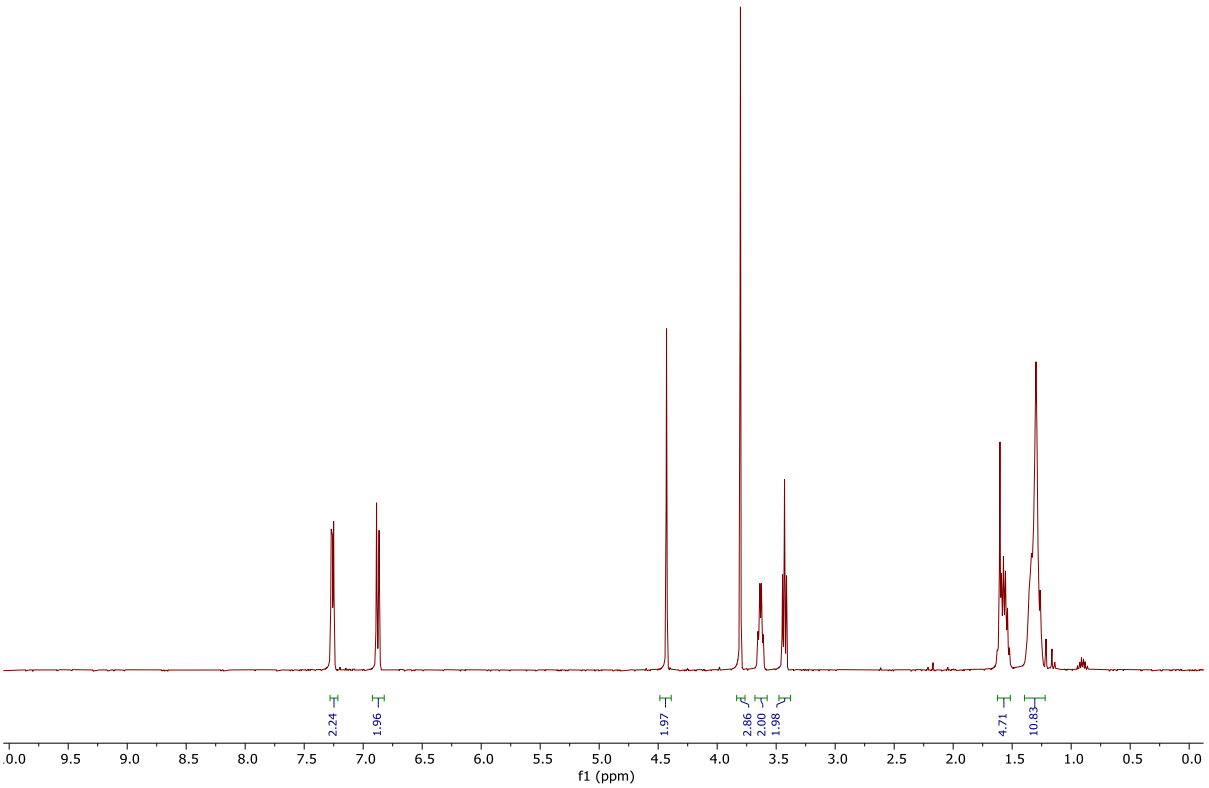


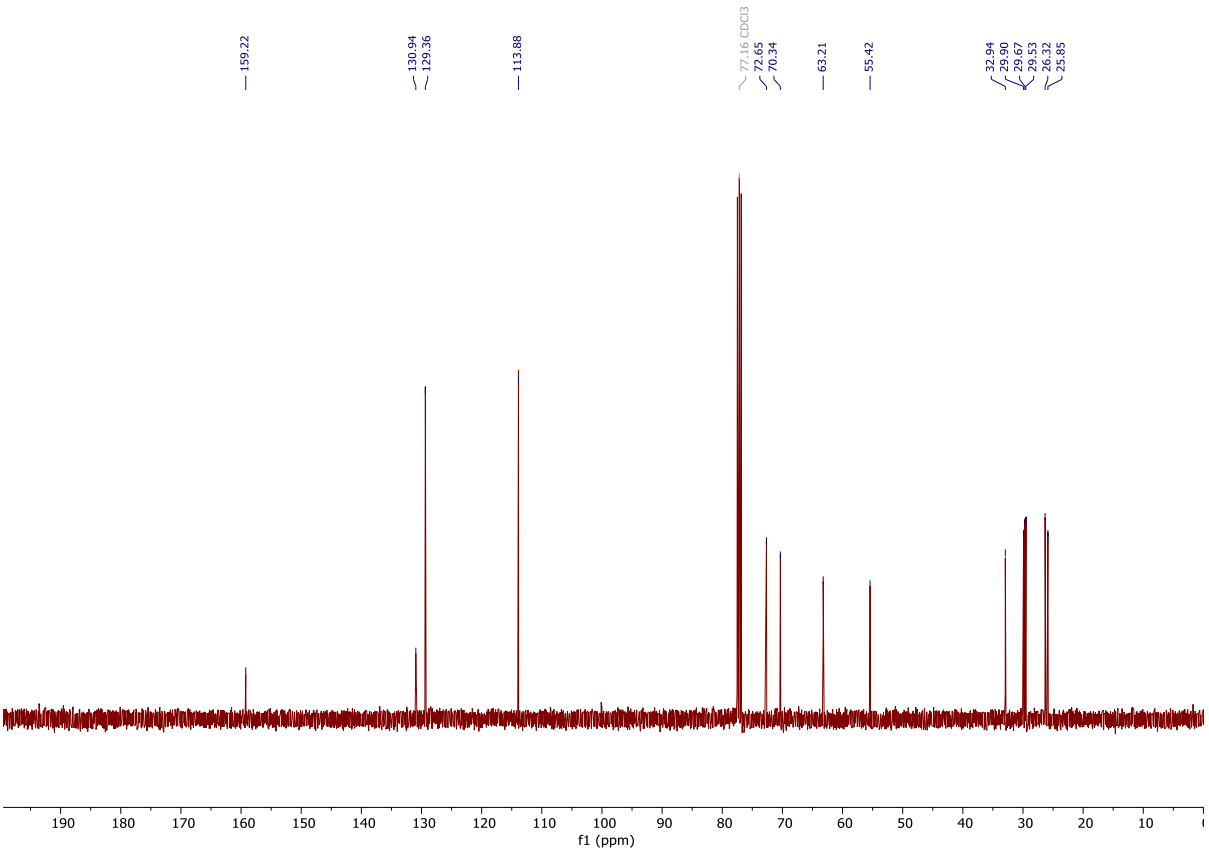


9-(4-Methoxybenzyloxy)nonan-1-ol 174

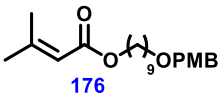


174

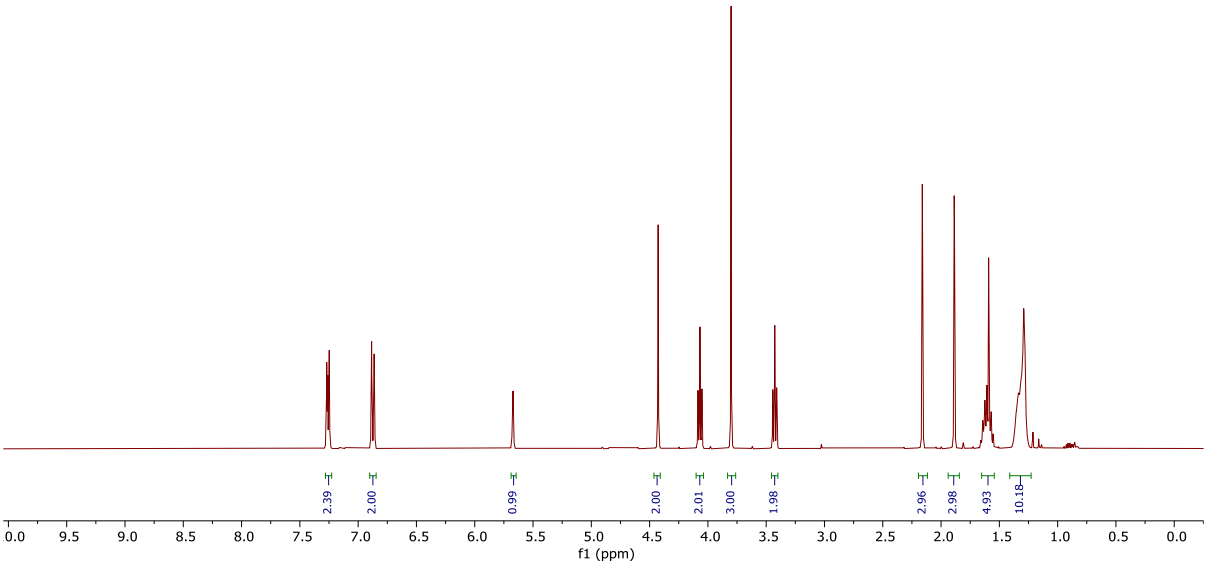


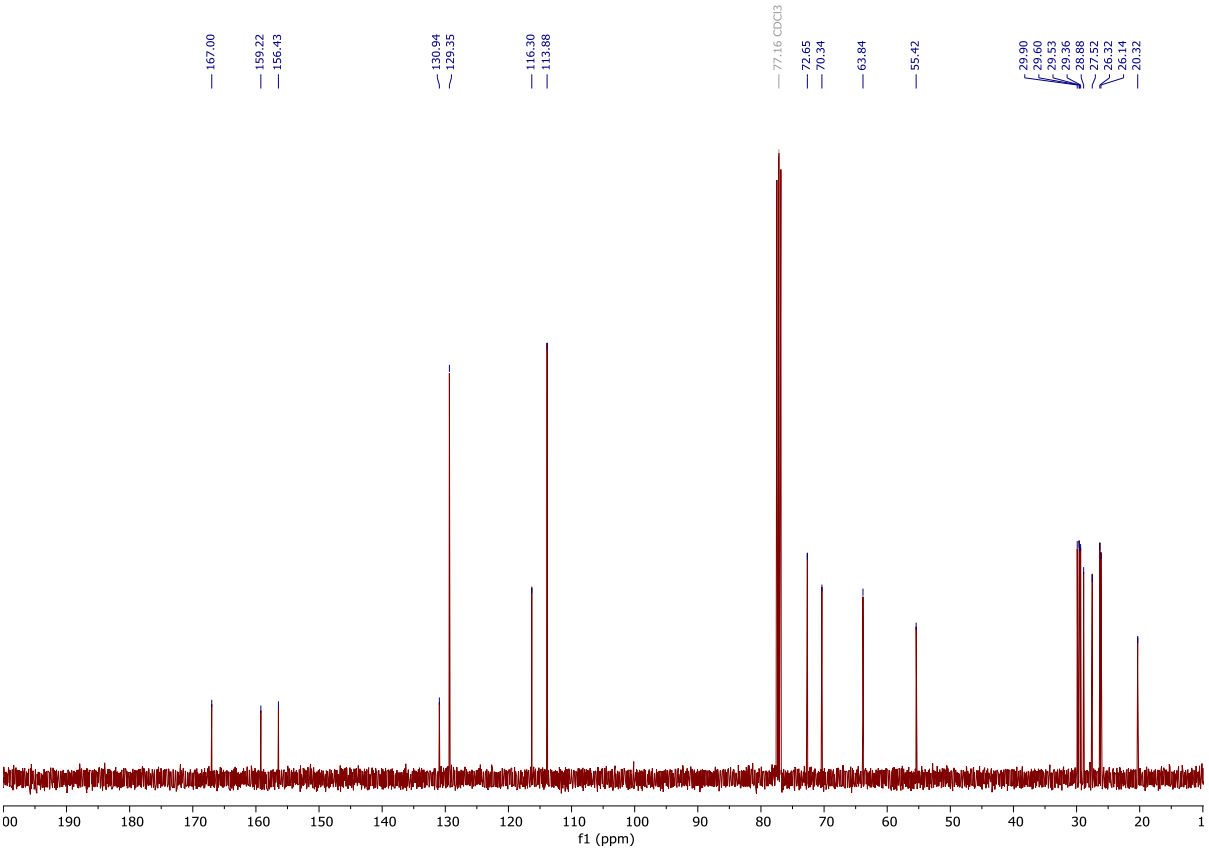


9-(4-Methoxybenzyloxy)nonyl 3-methylbut-2-enoate 176

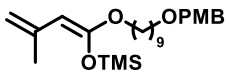


176

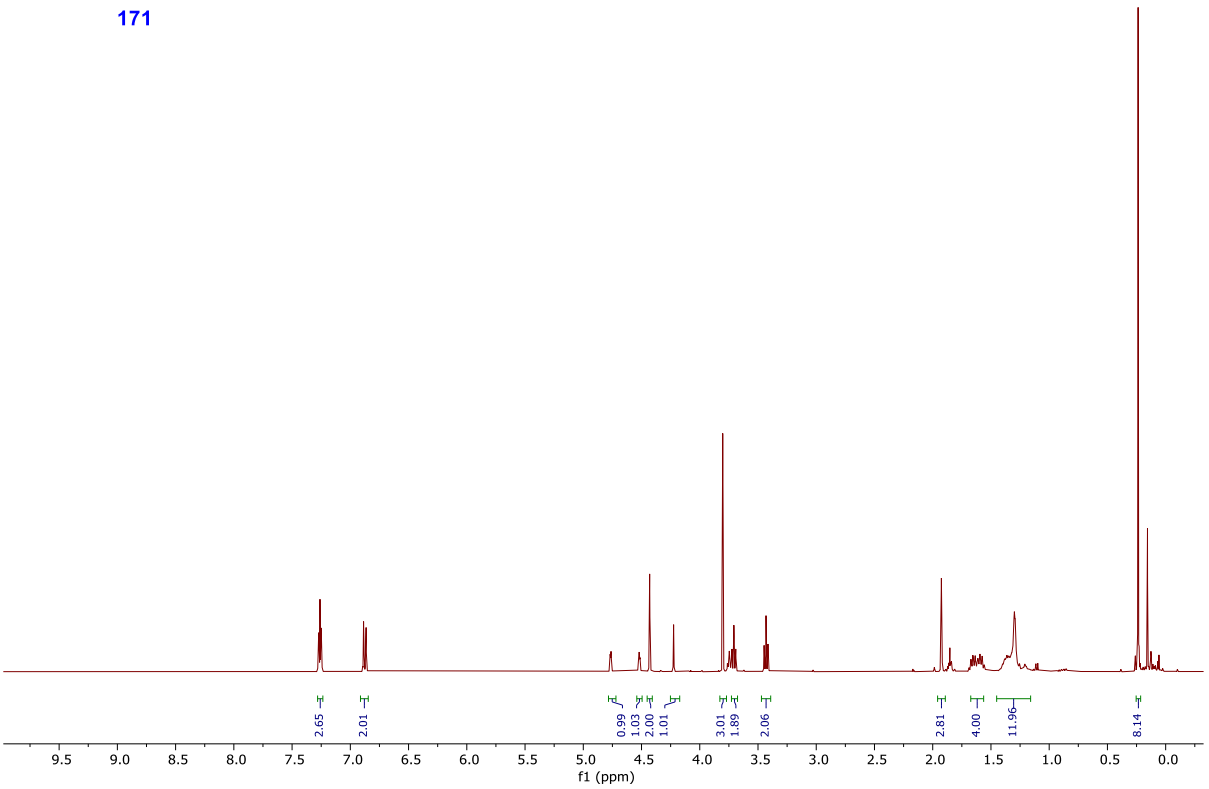


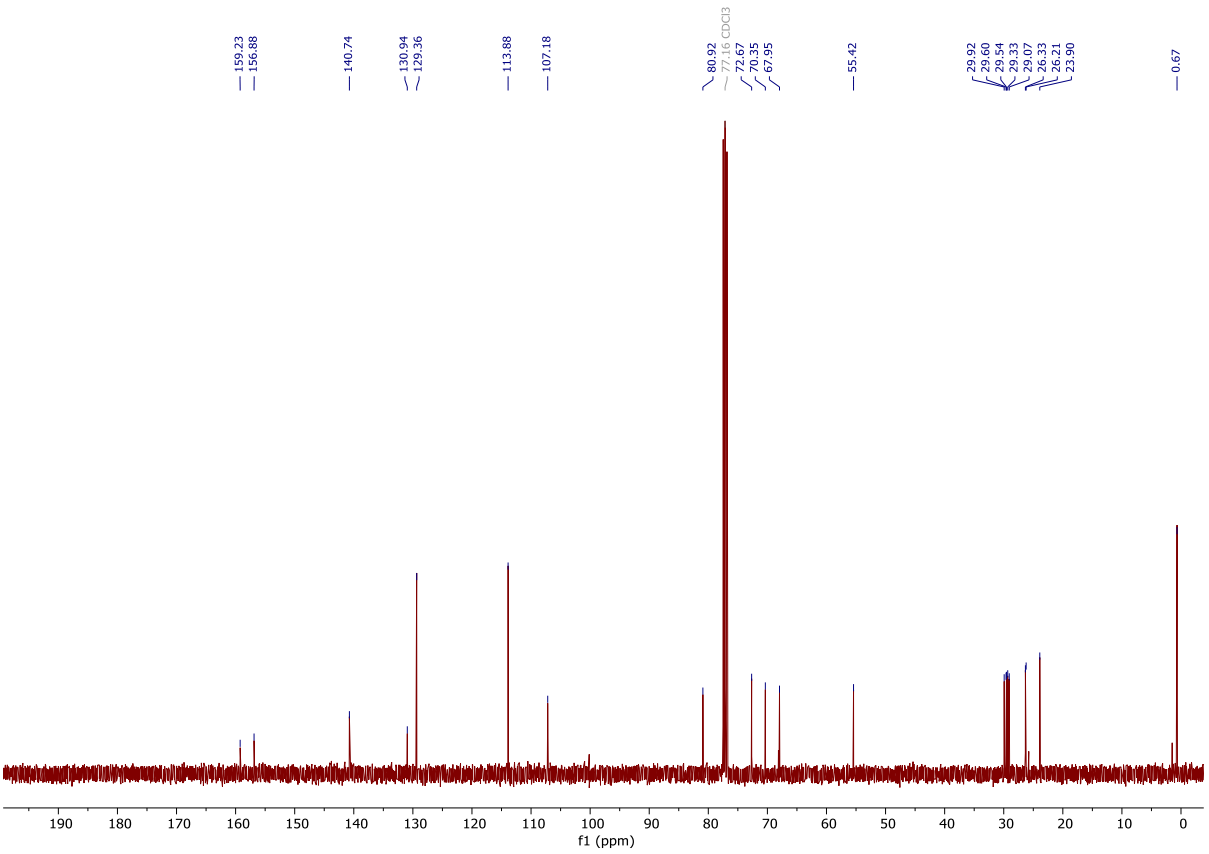


Silyl dienol ether 171

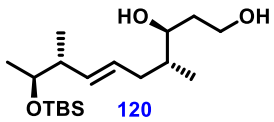


171

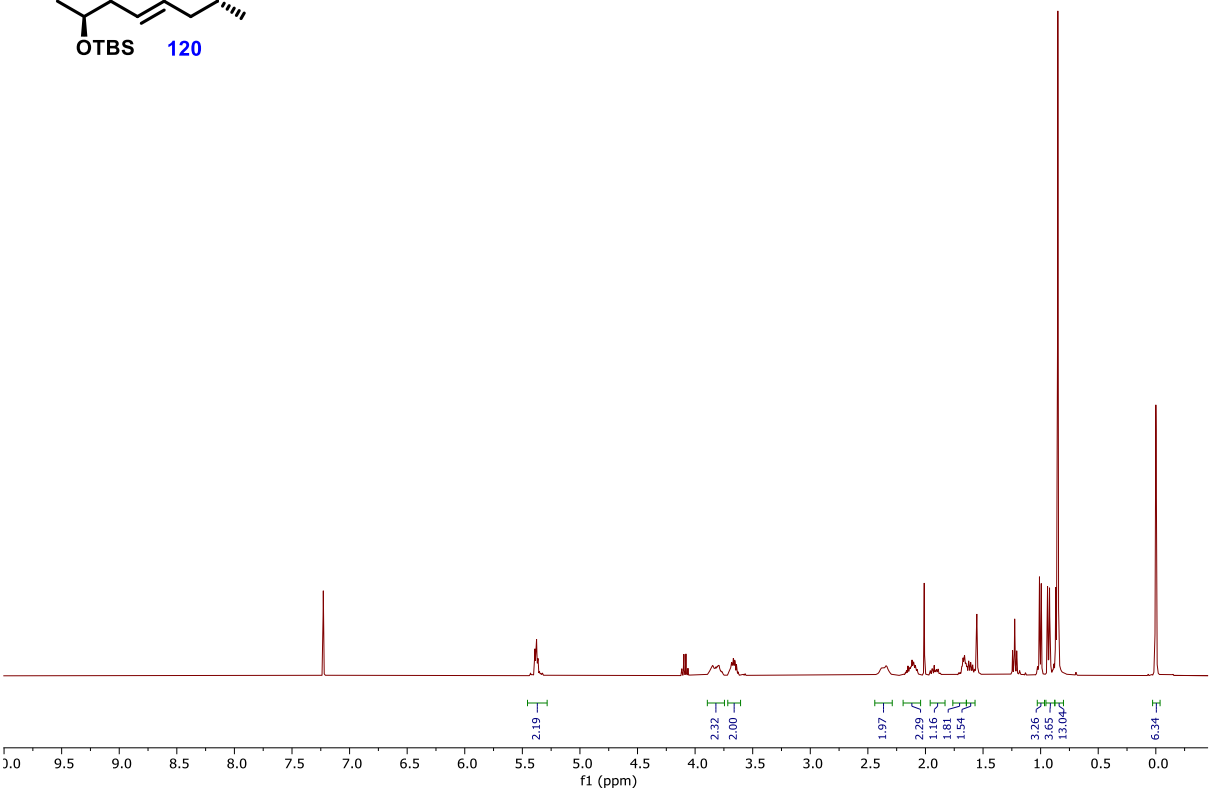


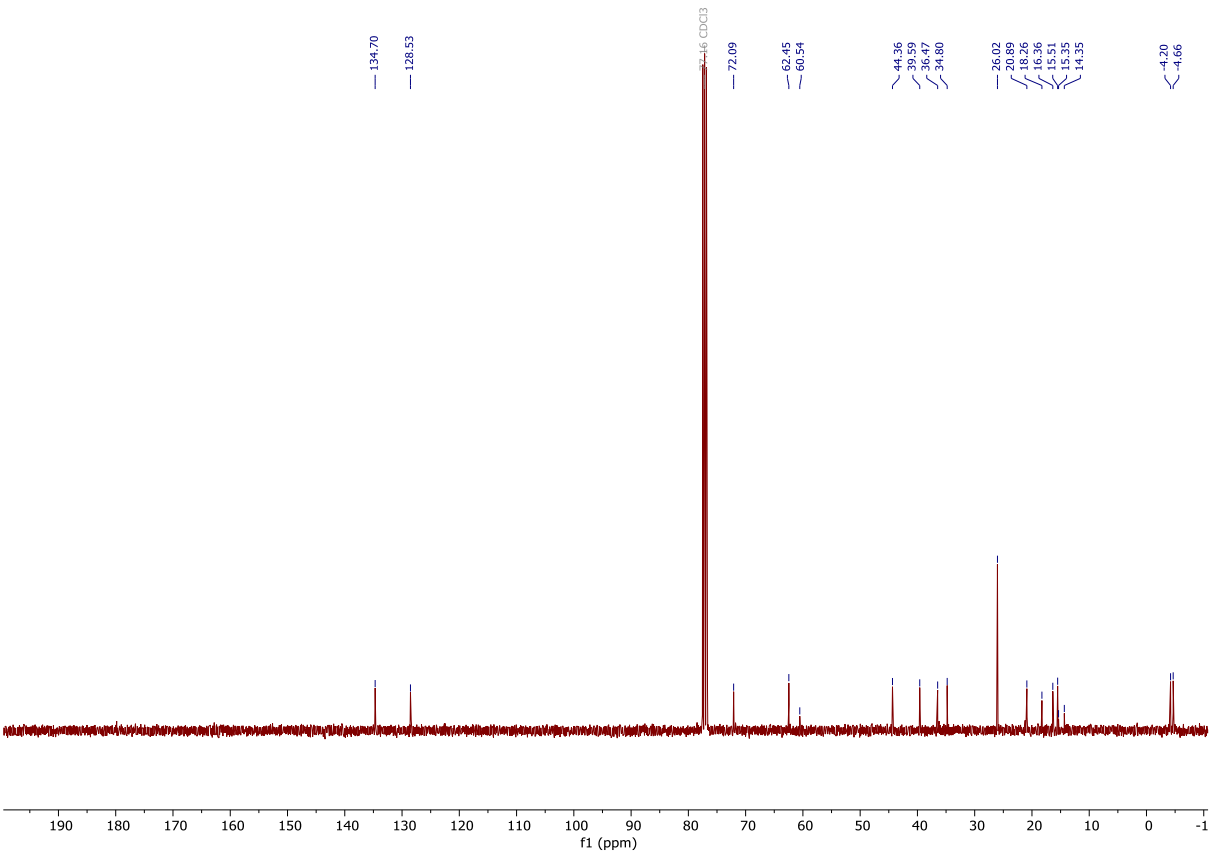


(3*S*,4*R*,8*R*,9*S*,*E*)-9-(*tert*-butyldimethylsilyloxy)-3-hydroxy-4,8-dimethyldec-6-en-1-ol 120

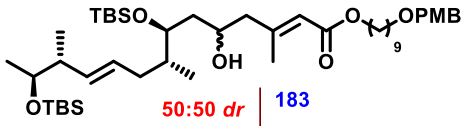


120



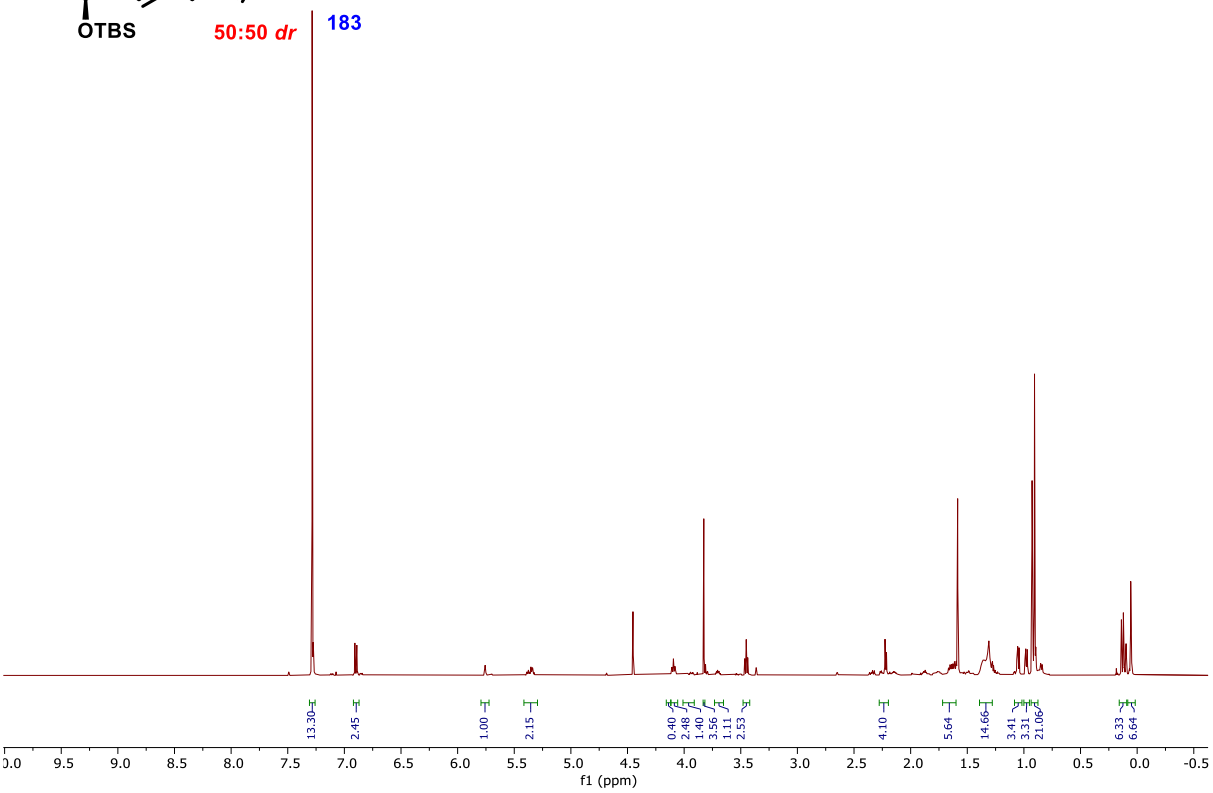


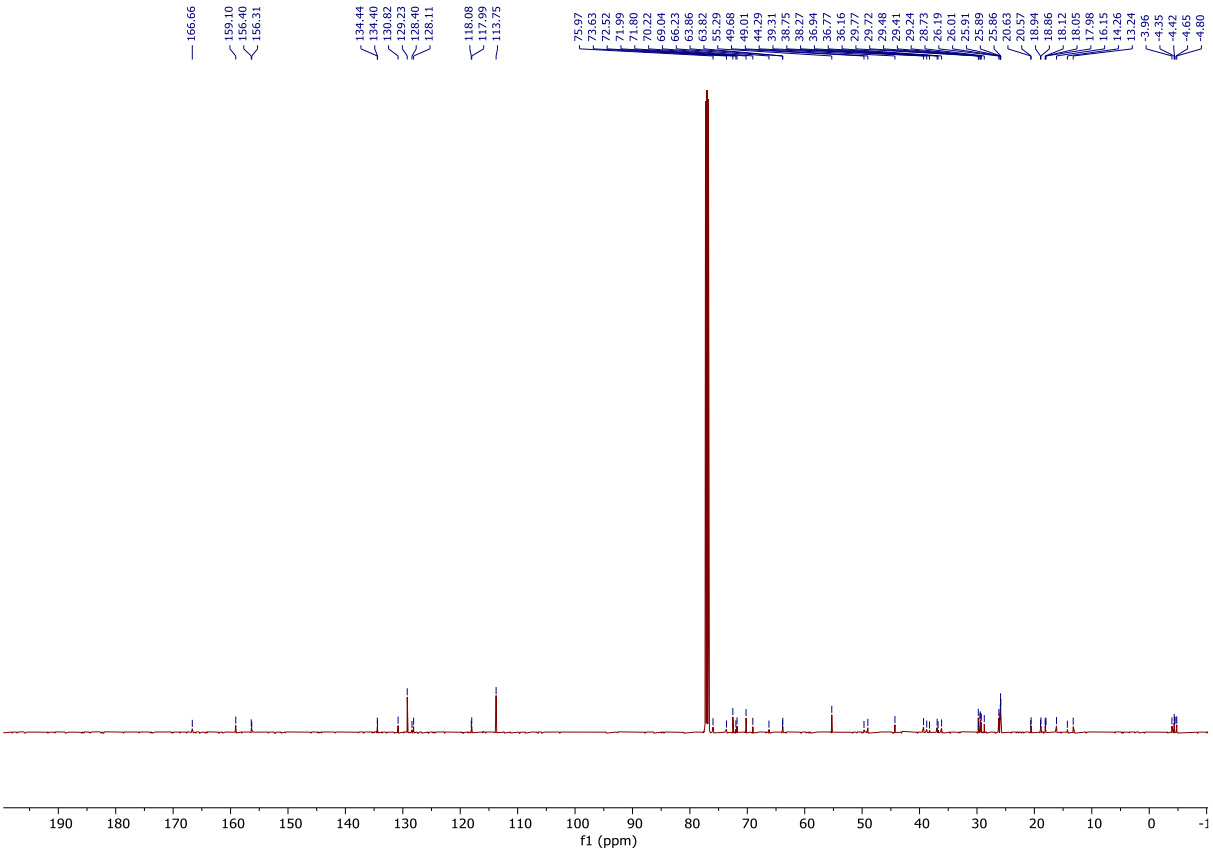
9-((4-Methoxybenzyl)oxy)nonyl (2*E*,7*S*,8*R*,10*E*,12*R*,13*S*)-7,13-bis((tert-butyldimethylsilyl)oxy)-5-hydroxy-3,8,12-trimethyltetradeca-2,10-dienoate 183



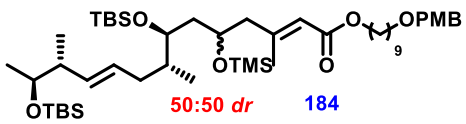
50:50 *dr*

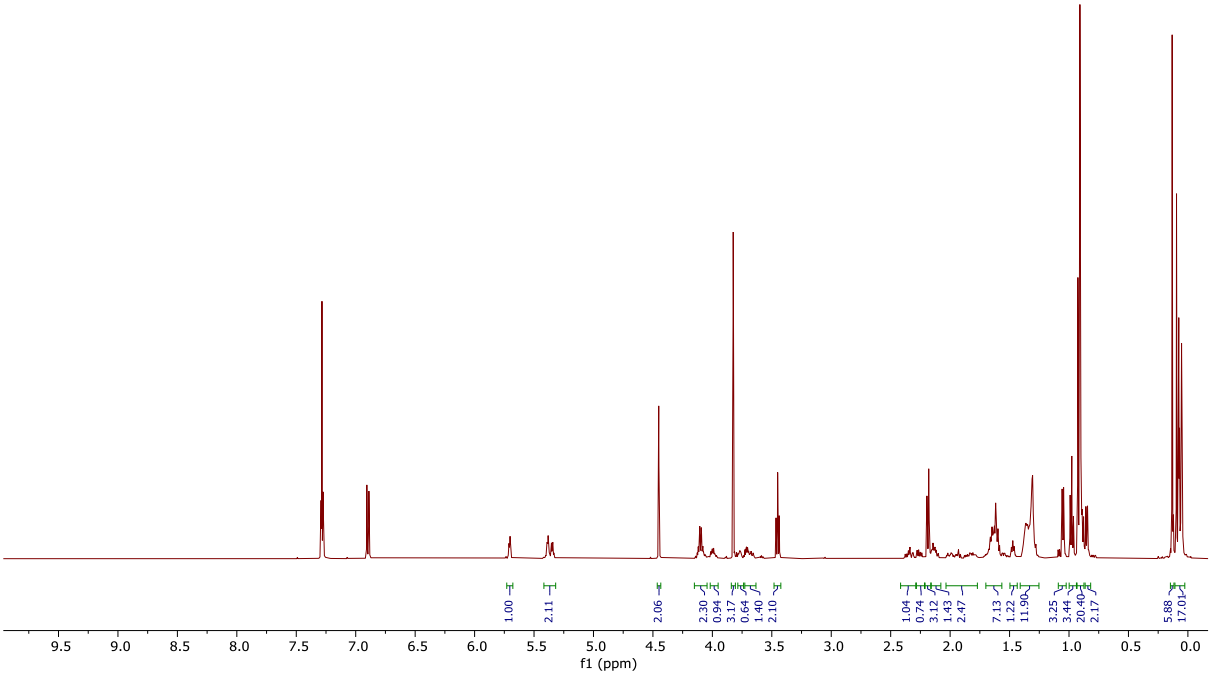
183

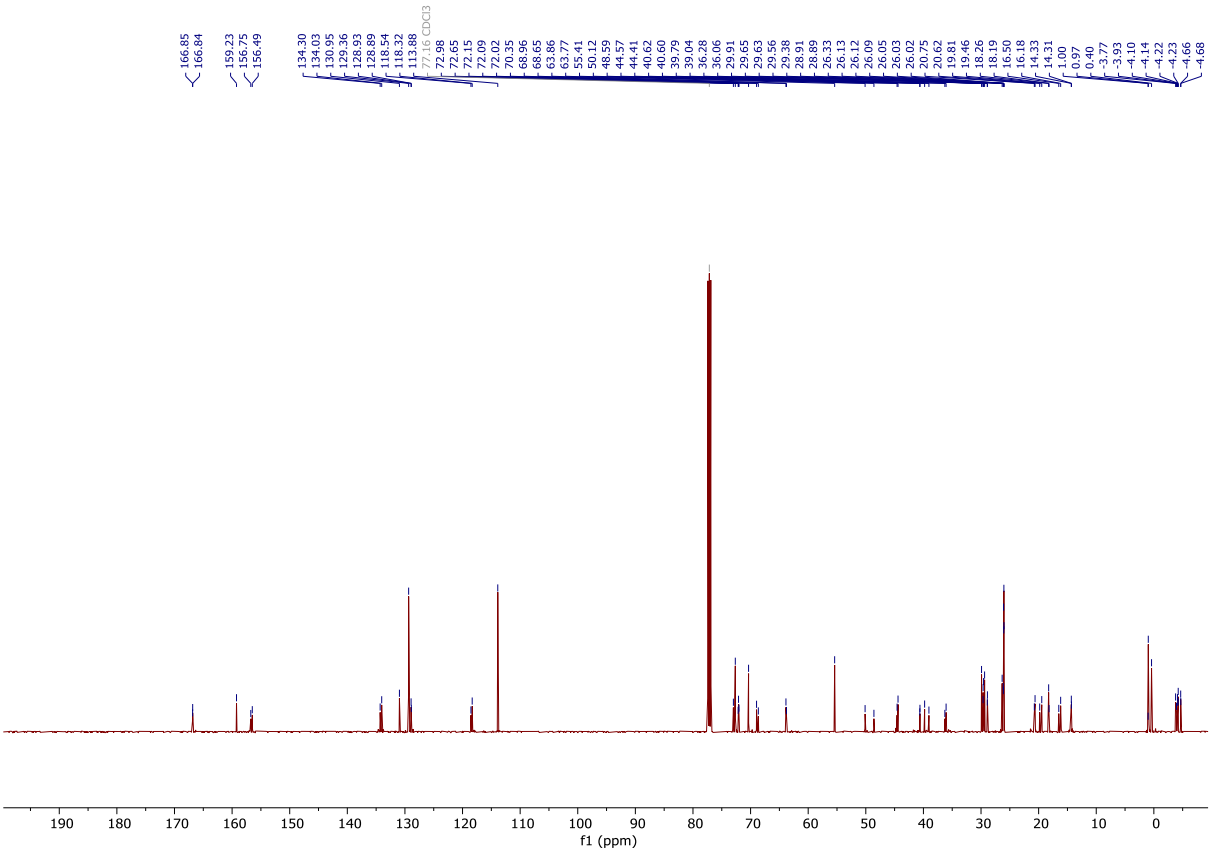




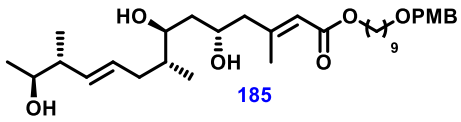
9-((4-Methoxybenzyl)oxy)nonyl (2*E*,7*S*,8*R*,10*E*,12*R*,13*S*)-7,13-bis((tert-butyldimethylsilyl)oxy)-3,8,12-trimethyl-5-((trimethylsilyl)oxy)tetradeca-2,10-dienoate 184



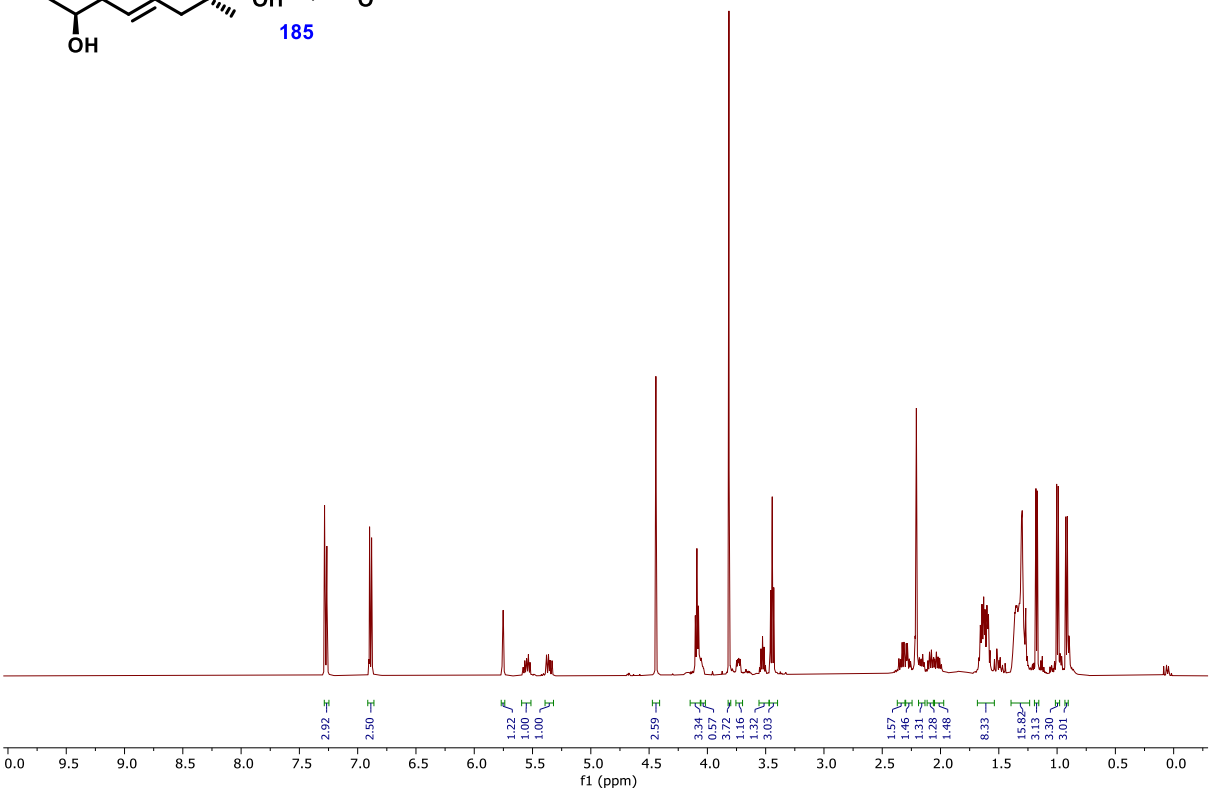


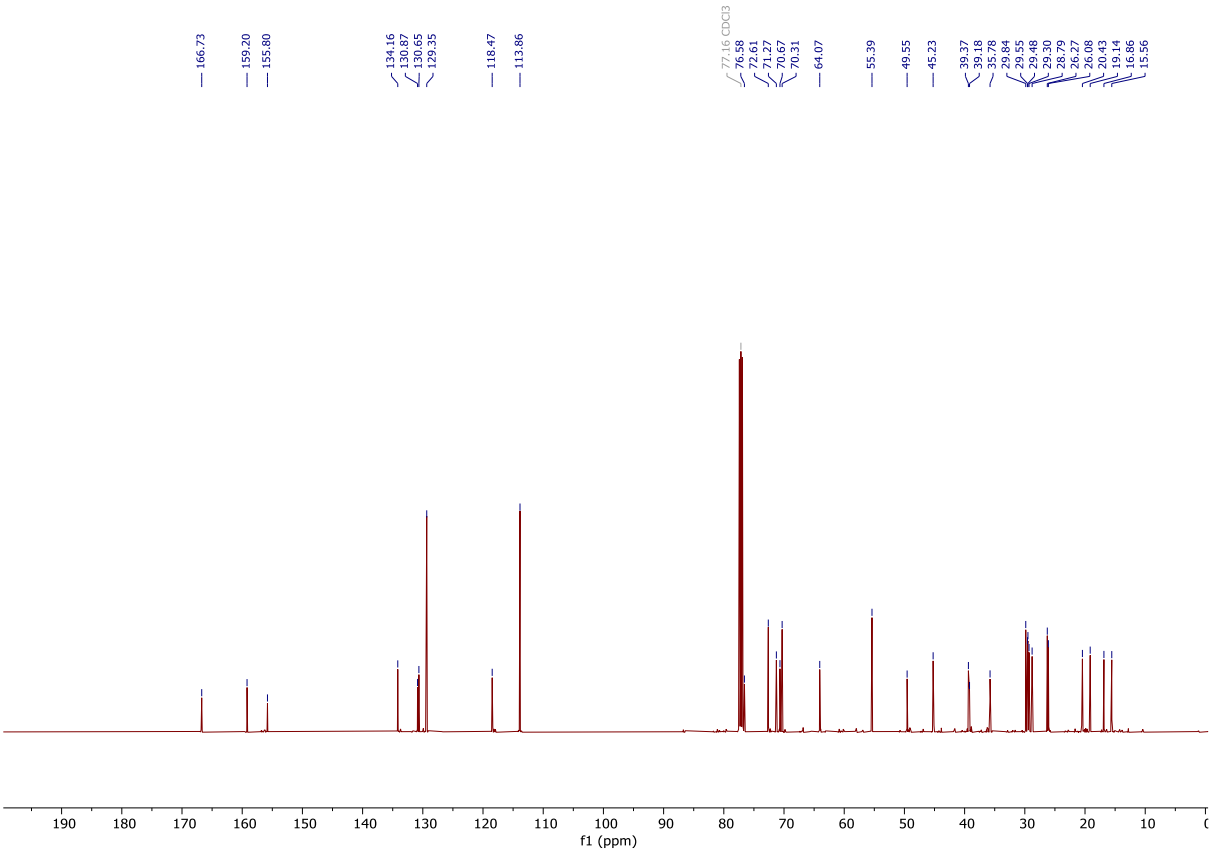


para-Methoxybenzyl ether of (_S_)-des-6-hydroxy-desepoxy Mupirocin W4-OH 185

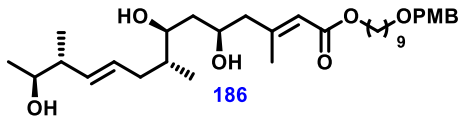


185

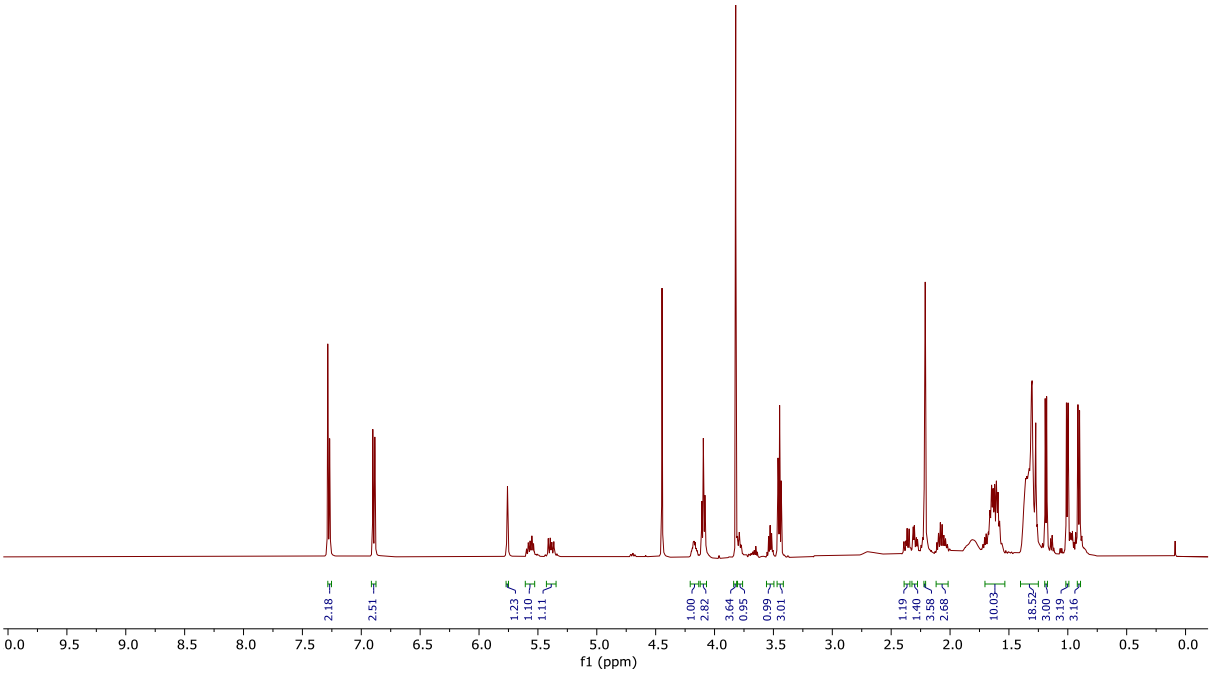


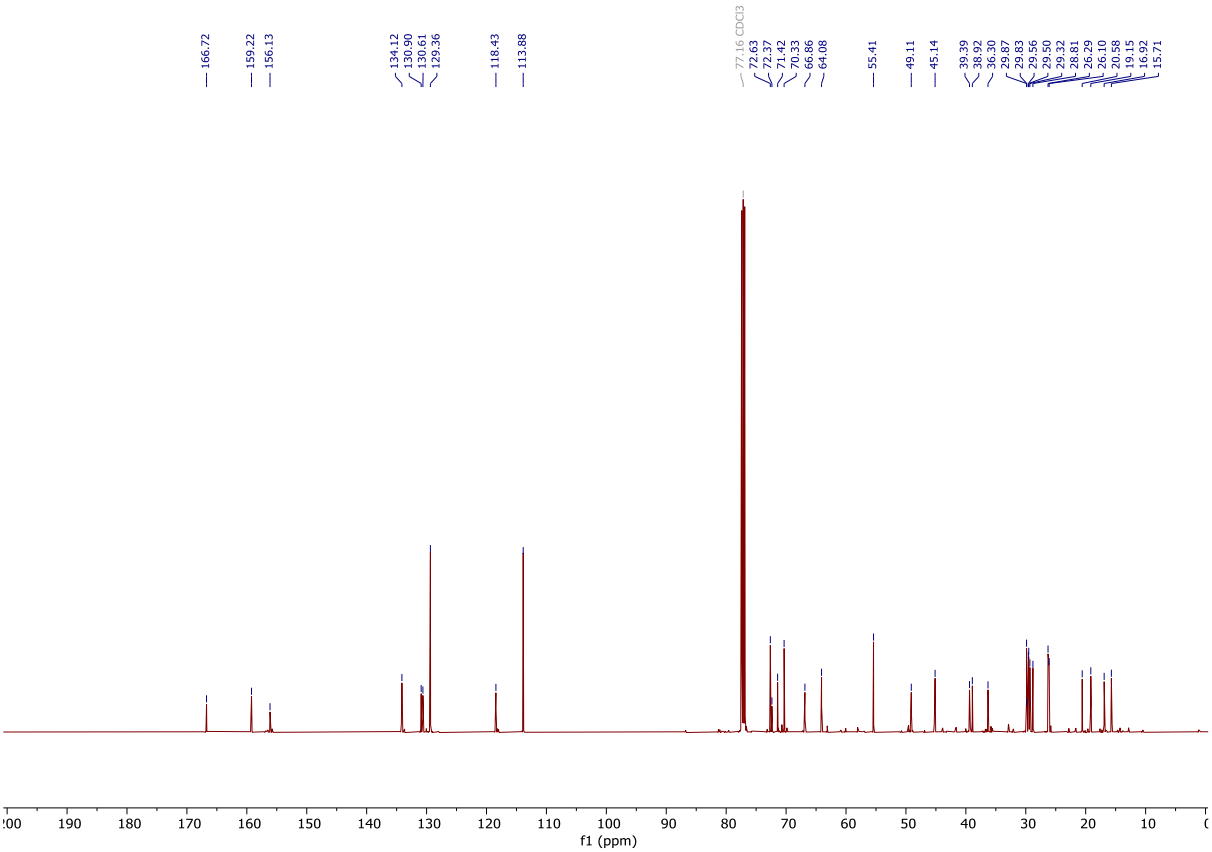


***para*-Methoxybenzyl ether of (*R*)-des-6-hydroxy-desepoxy Mupirocin W4-OH 186**

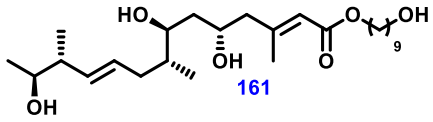


186

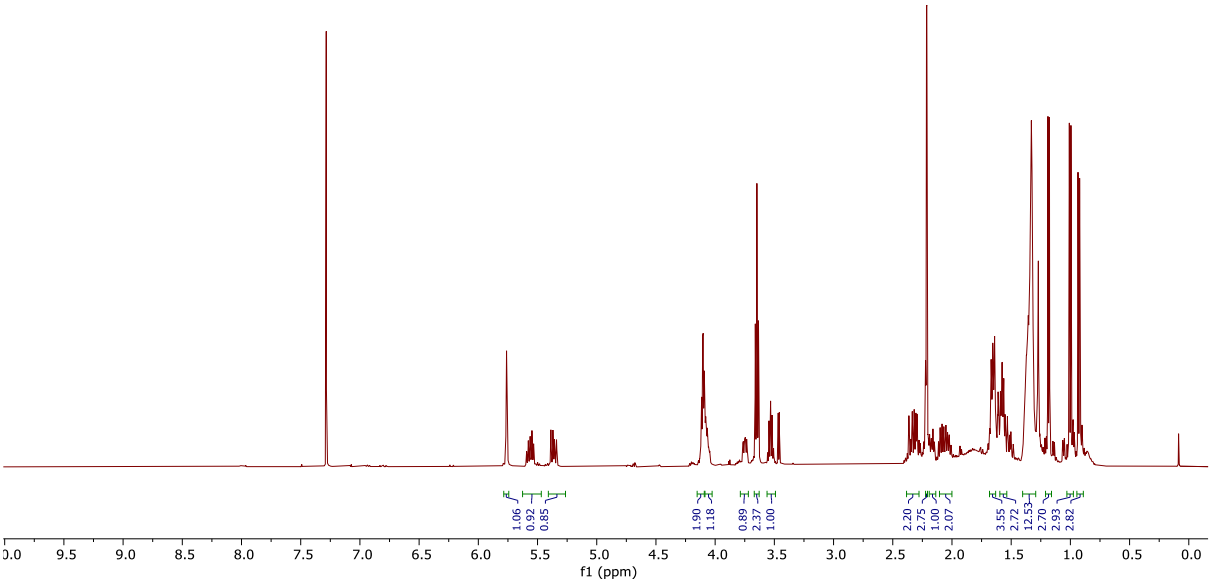


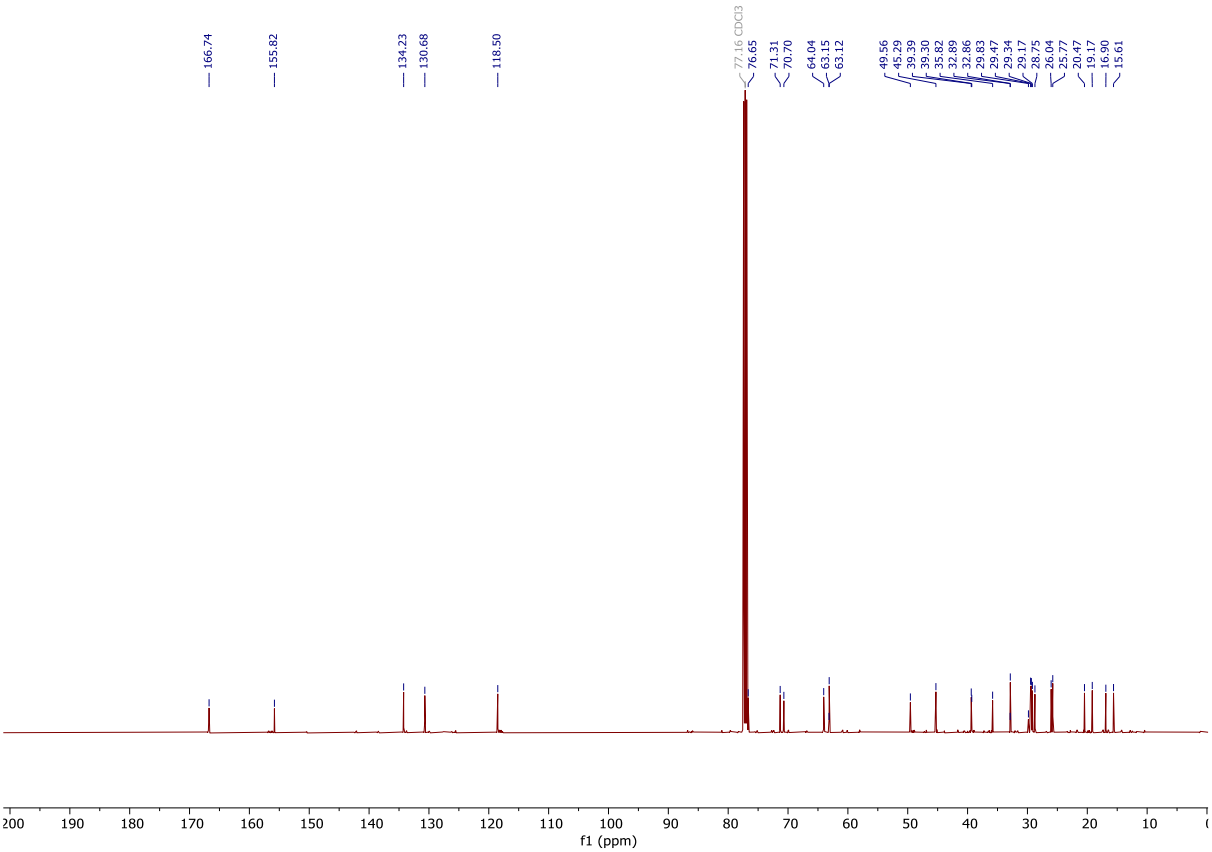


(*S*)-Des-6-hydroxy-desepoxy mupirocin W4-OH 161

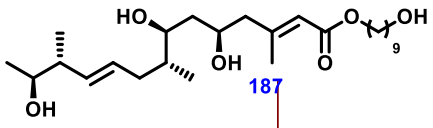


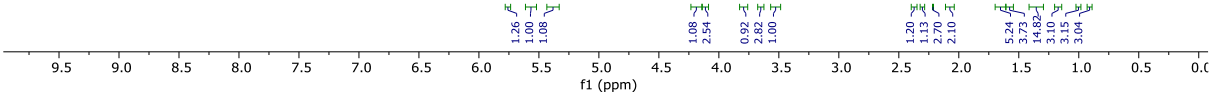
161

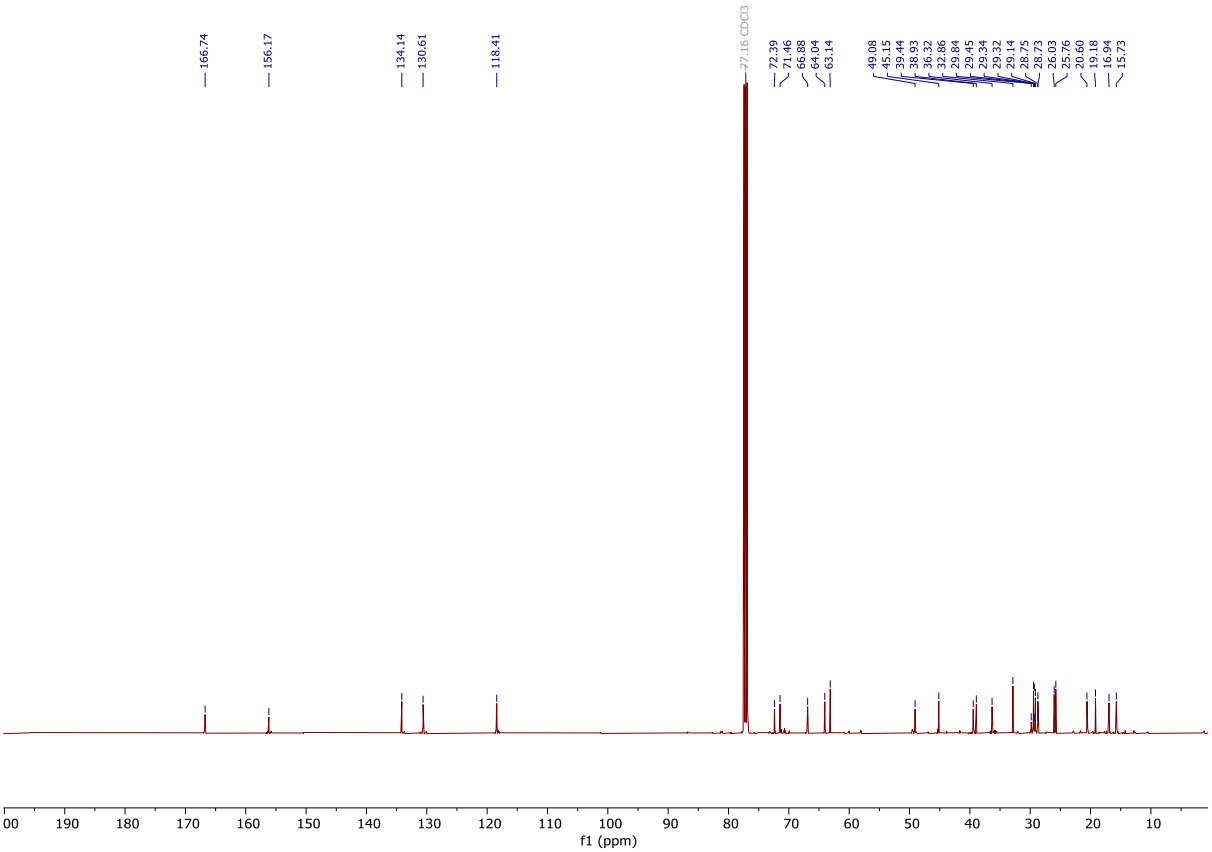




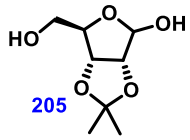
(*R*)-Des-6-hydroxy-desepoxy mupirocin W4-OH 187

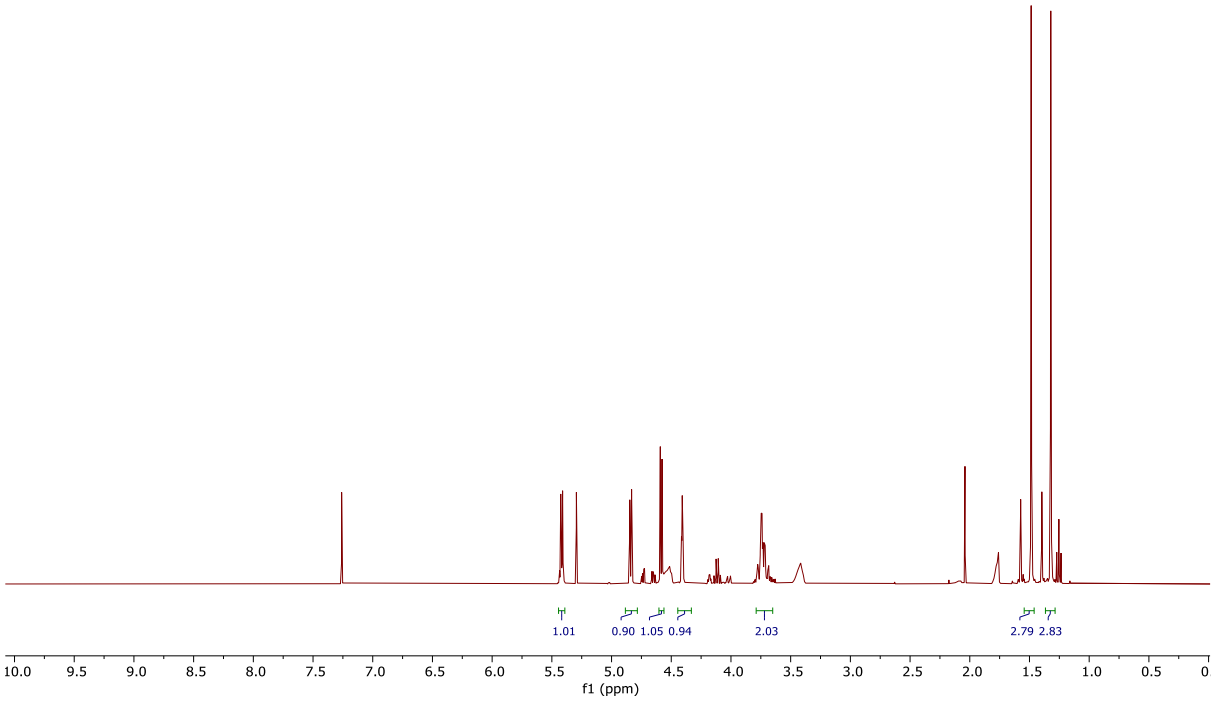


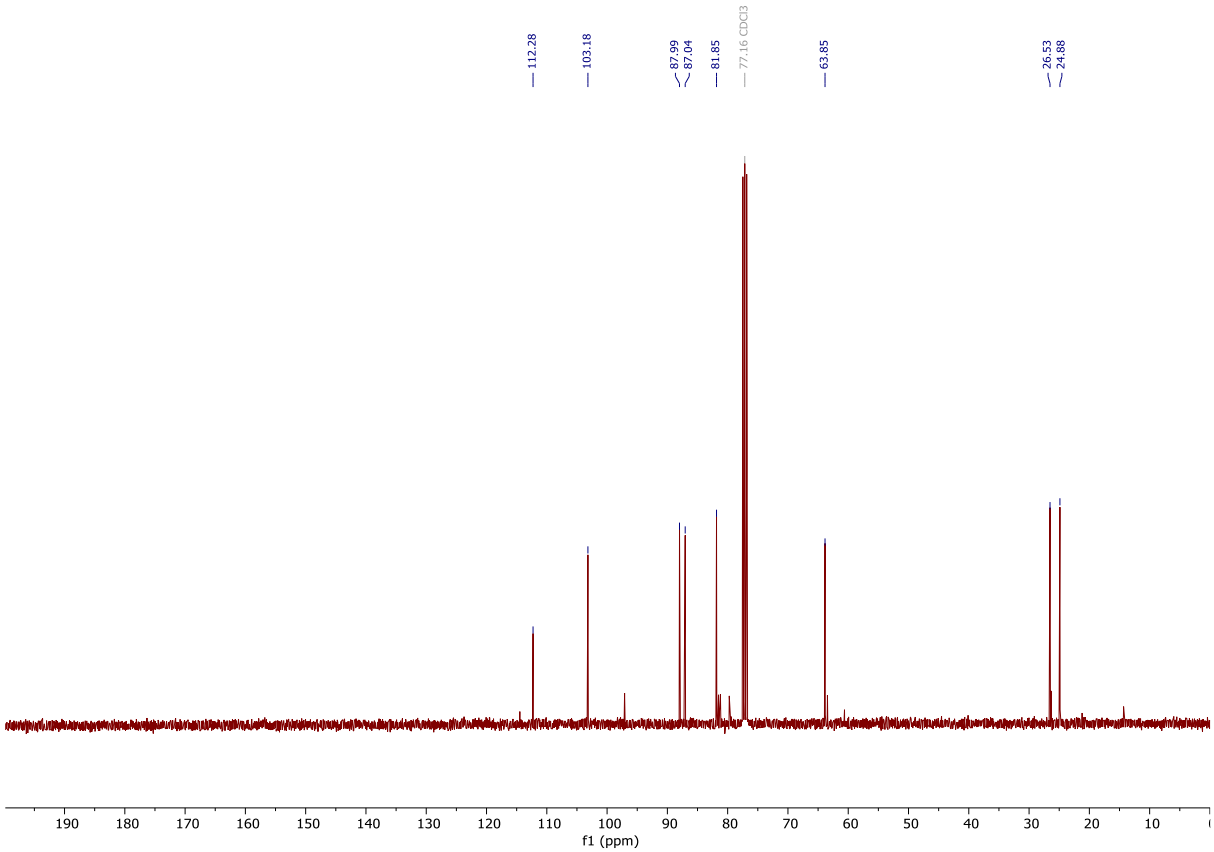




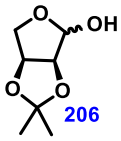
(2*S*,3*S*)-Acetonide of (3*R*,4*S*,5*R*)-5-(hydroxymethyl)tetrahydrofuran-2,3,4-triol 205

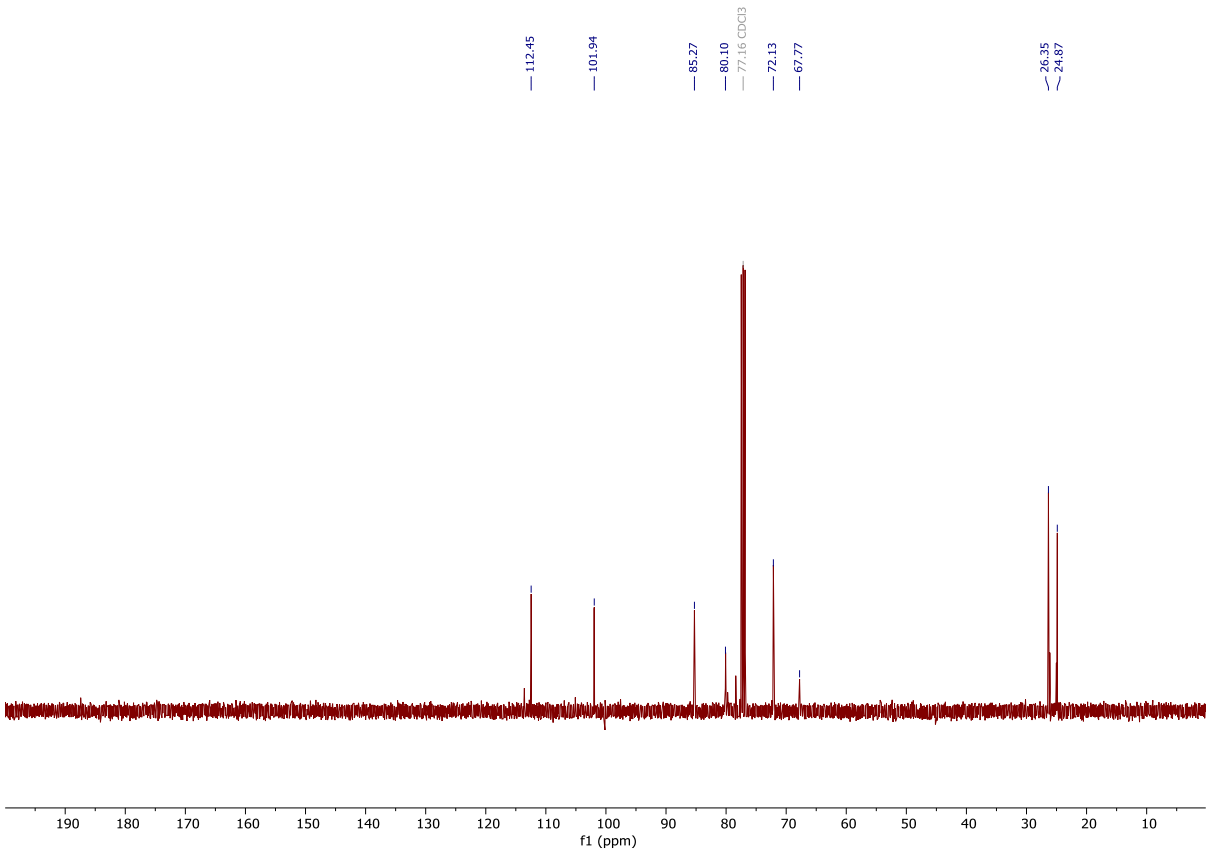




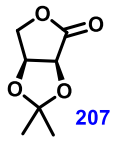


(2*S*,3*S*)-Acetonide of (3*S*,4*S*)-tetrahydrofuran-2,3,4-triol 206

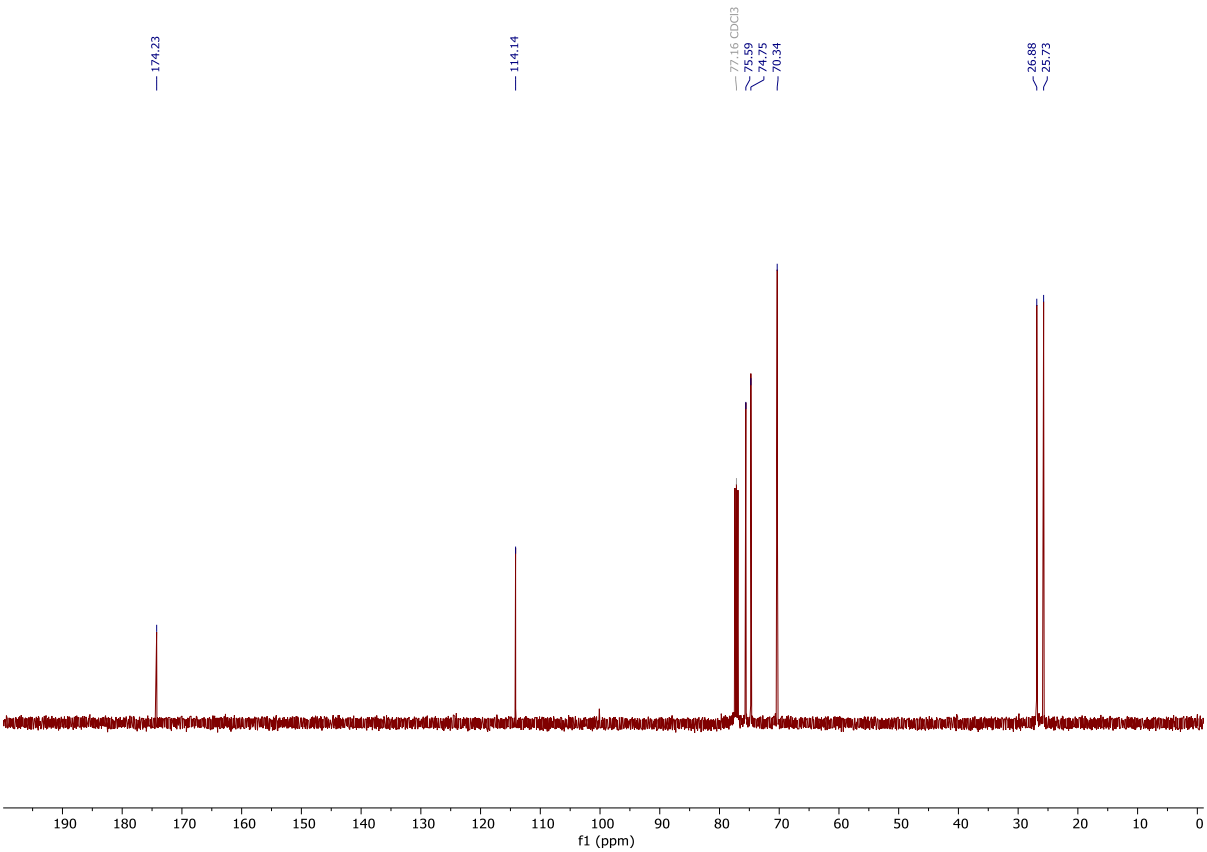




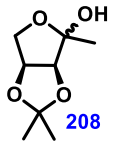
(2*S*,3*S*)-Acetonide of L-erythrono-1,4-lactone 207

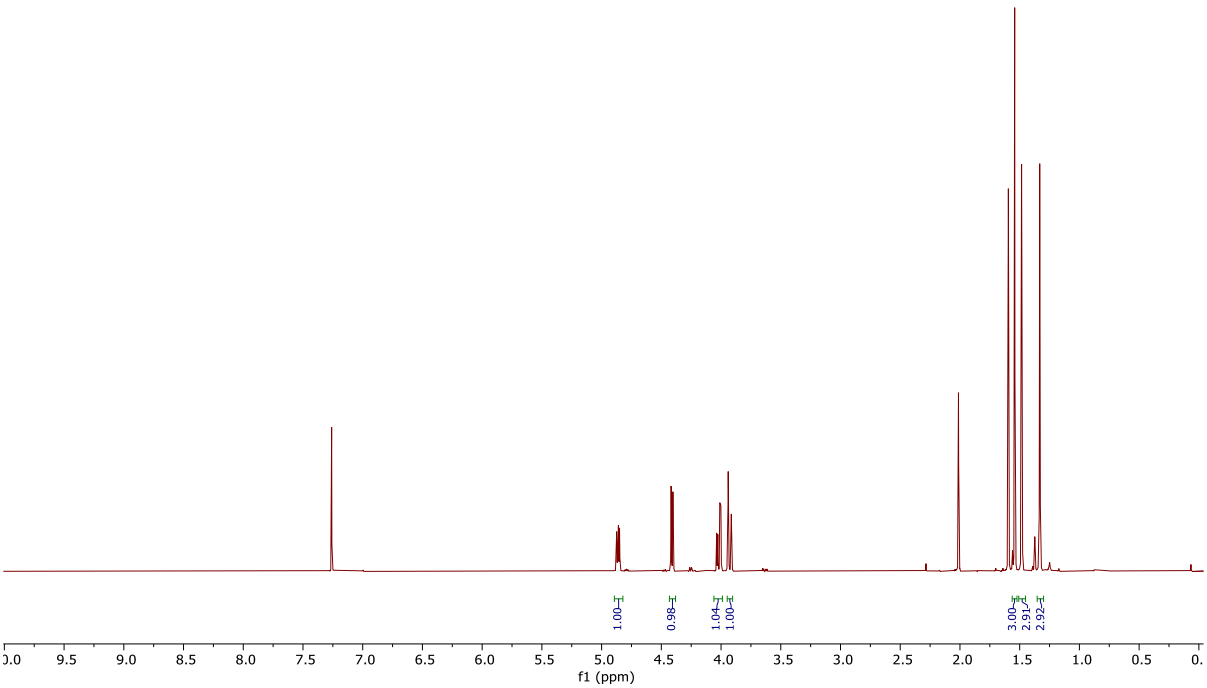


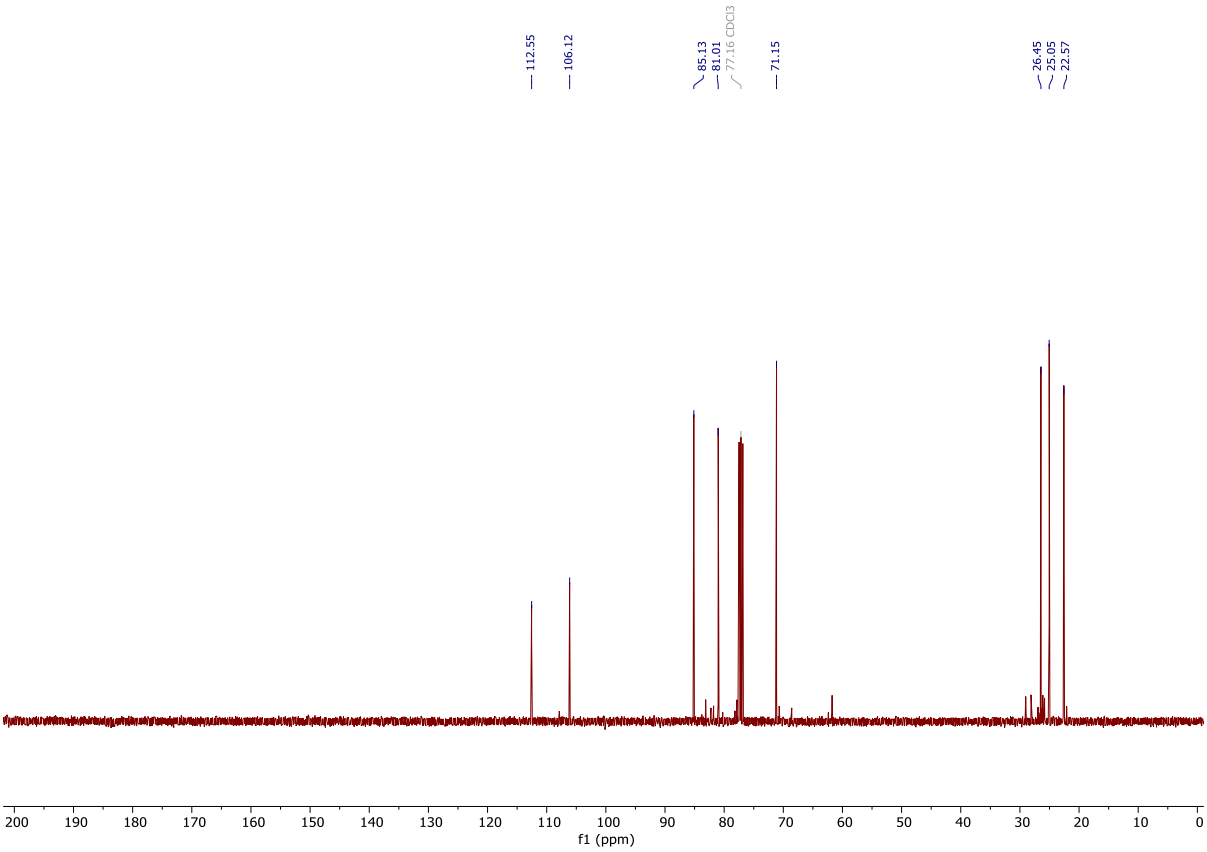
207



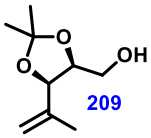
(2*S*,3*S*)-Acetonide of 1-methyltetrahydrofuran-1-ol 208



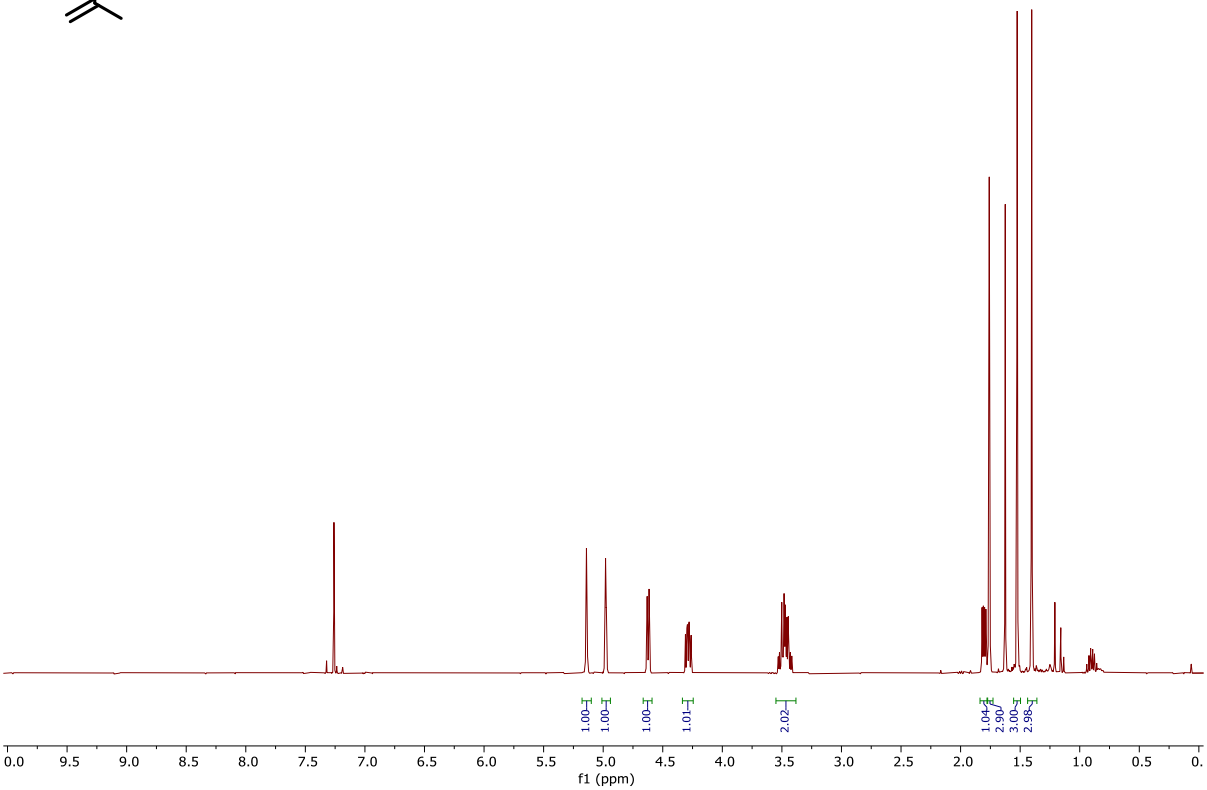


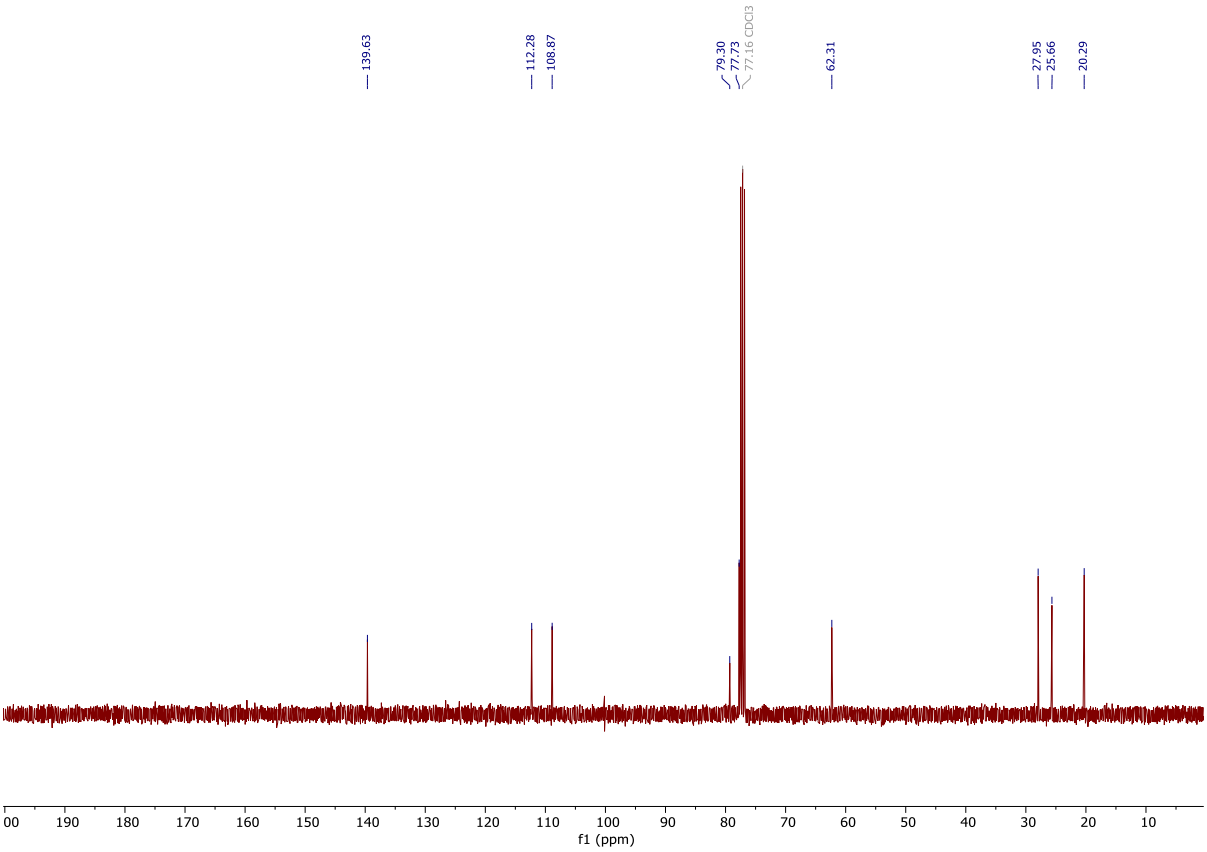


(2*S*,3*R*)-Acetonide of 4-methylpent-4-en-1-ol 209

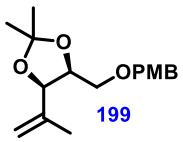


209

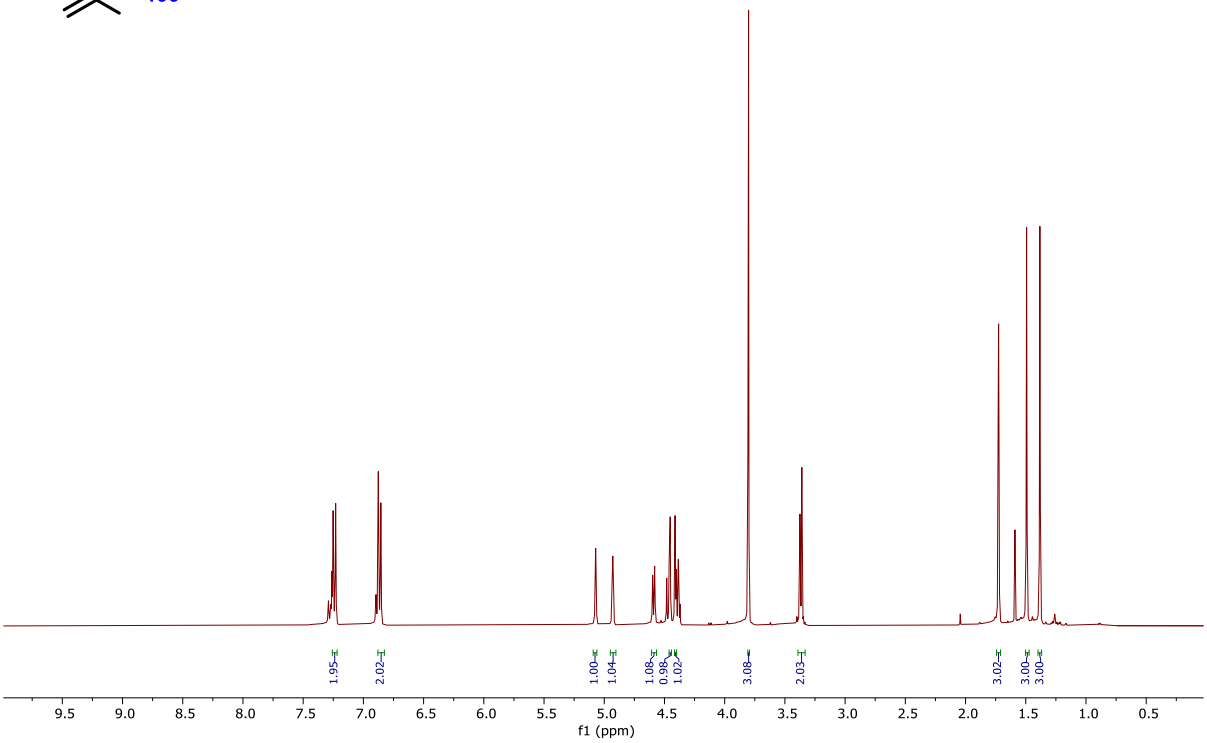


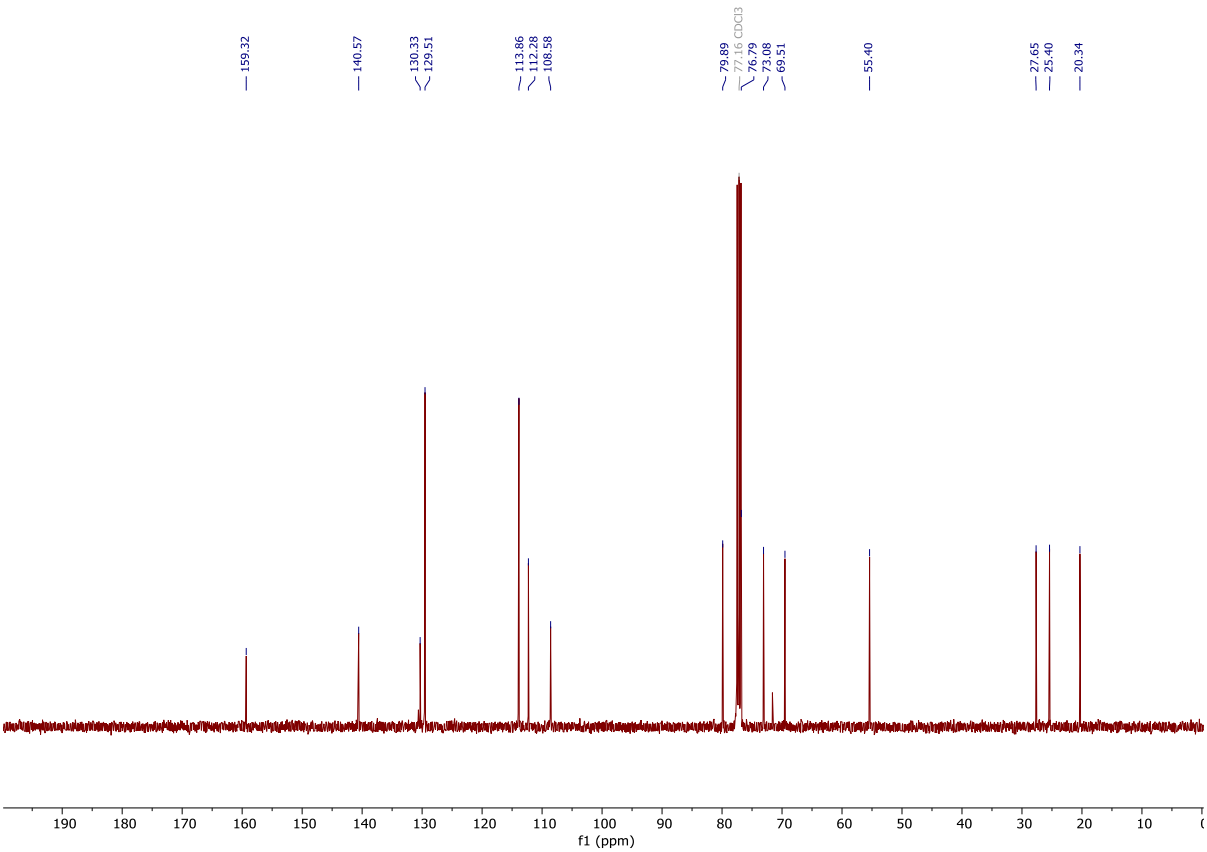


(2*S*,3*R*)-Acetonide of 1-(4-methoxybenzyloxy)-4-methylpent-4-ene 199

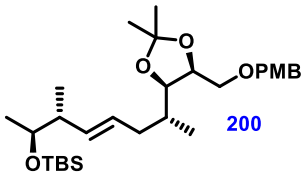


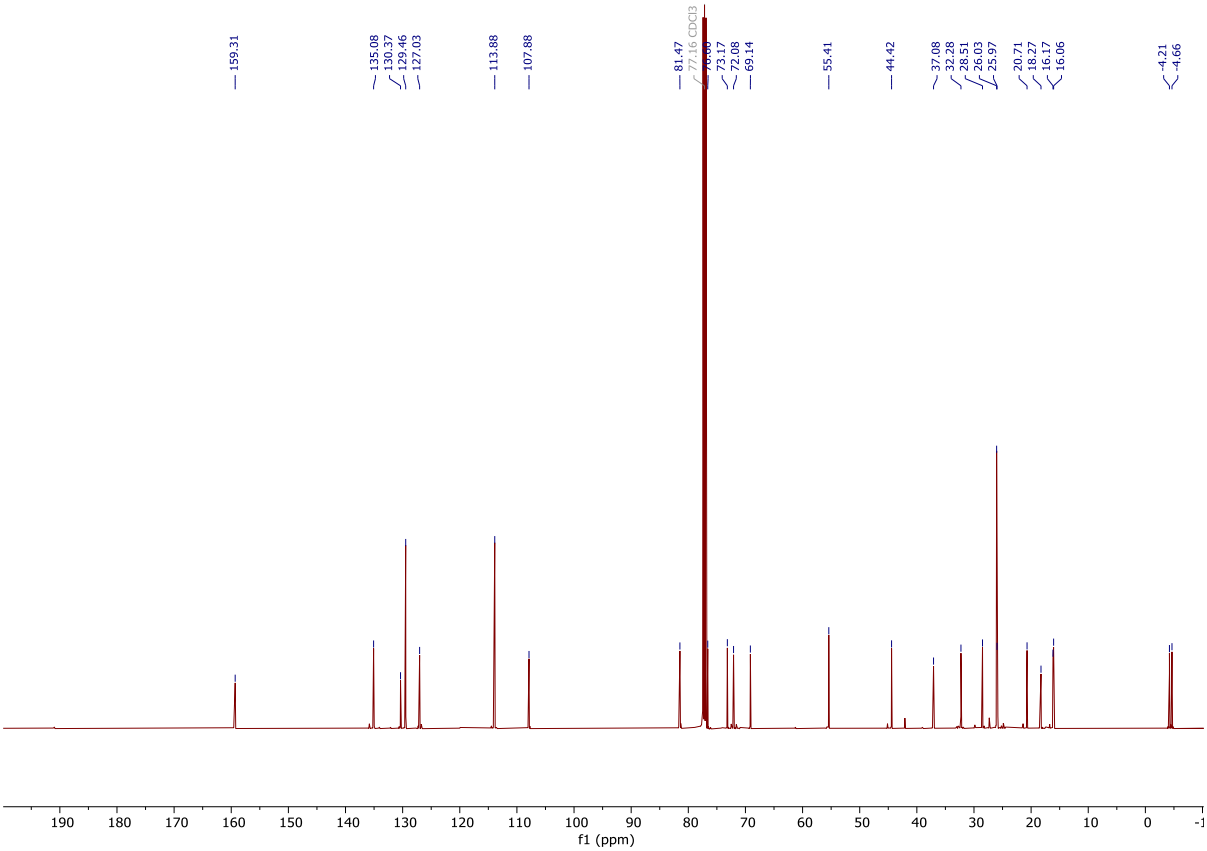
199



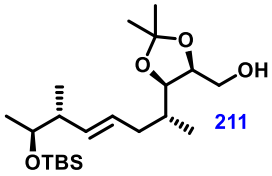


(8*R*,9*S*)-Acetonide of (2*S*,3*R*,7*R*,4*E*)-2-(*tert*-butyldimethylsilyloxy)-3,7-dimethyl-10-(4-methoxybenzyloxy)dec-4-ene 200

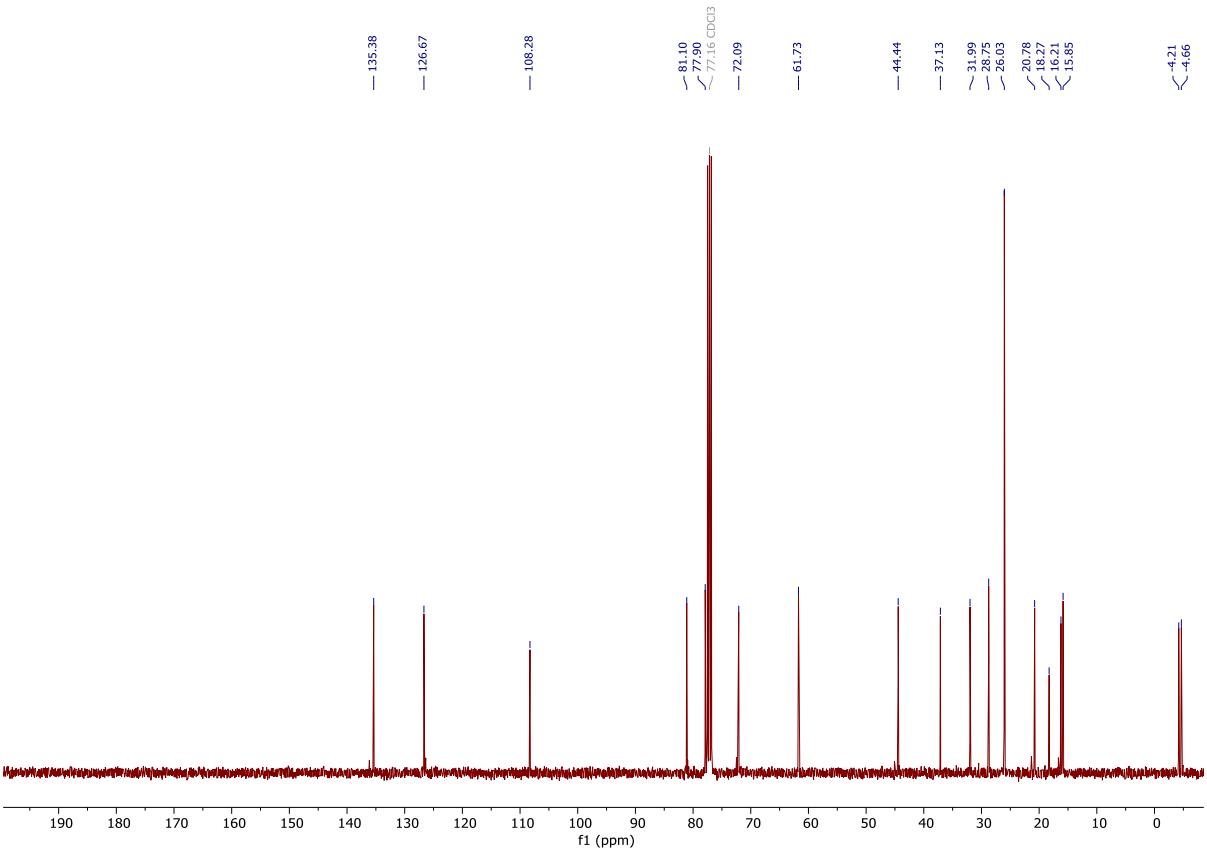


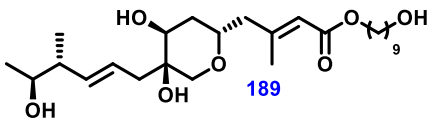


(8*R*,9*S*)-Acetonide of (2*S*,3*R*,7*R*,4*E*)-2-(*tert*-butyldimethylsilyloxy)-3,7-dimethyldec-4-ene 211

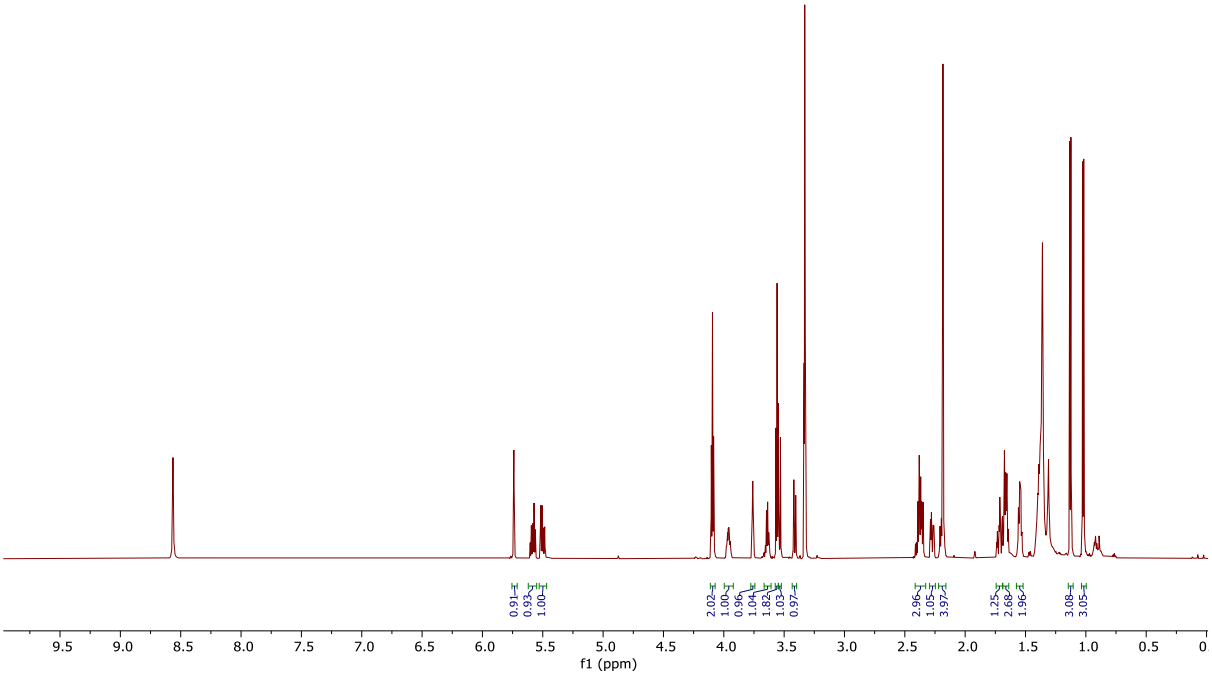


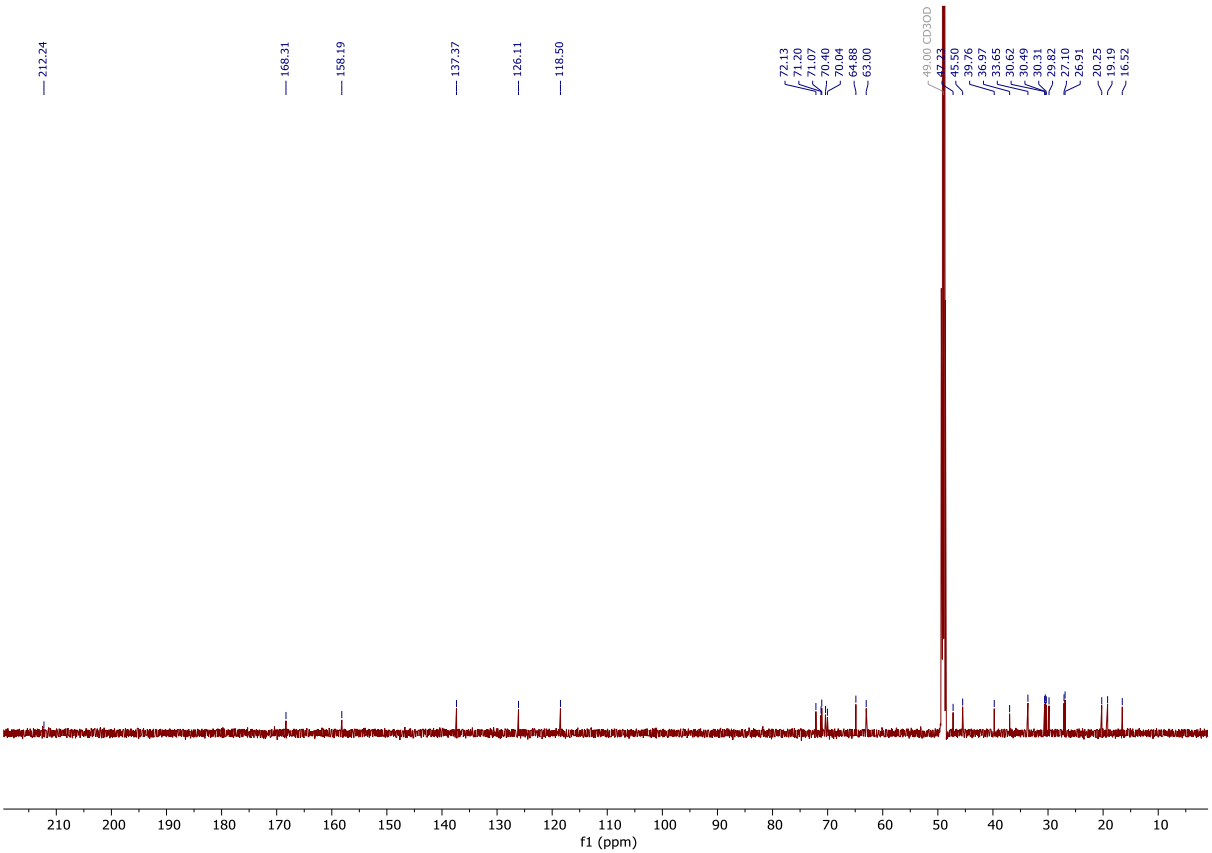
211

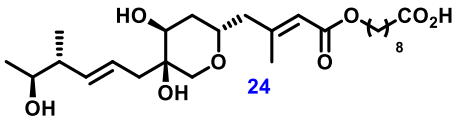



HO
OH
O
O
OH
9
O
OH
OH

189

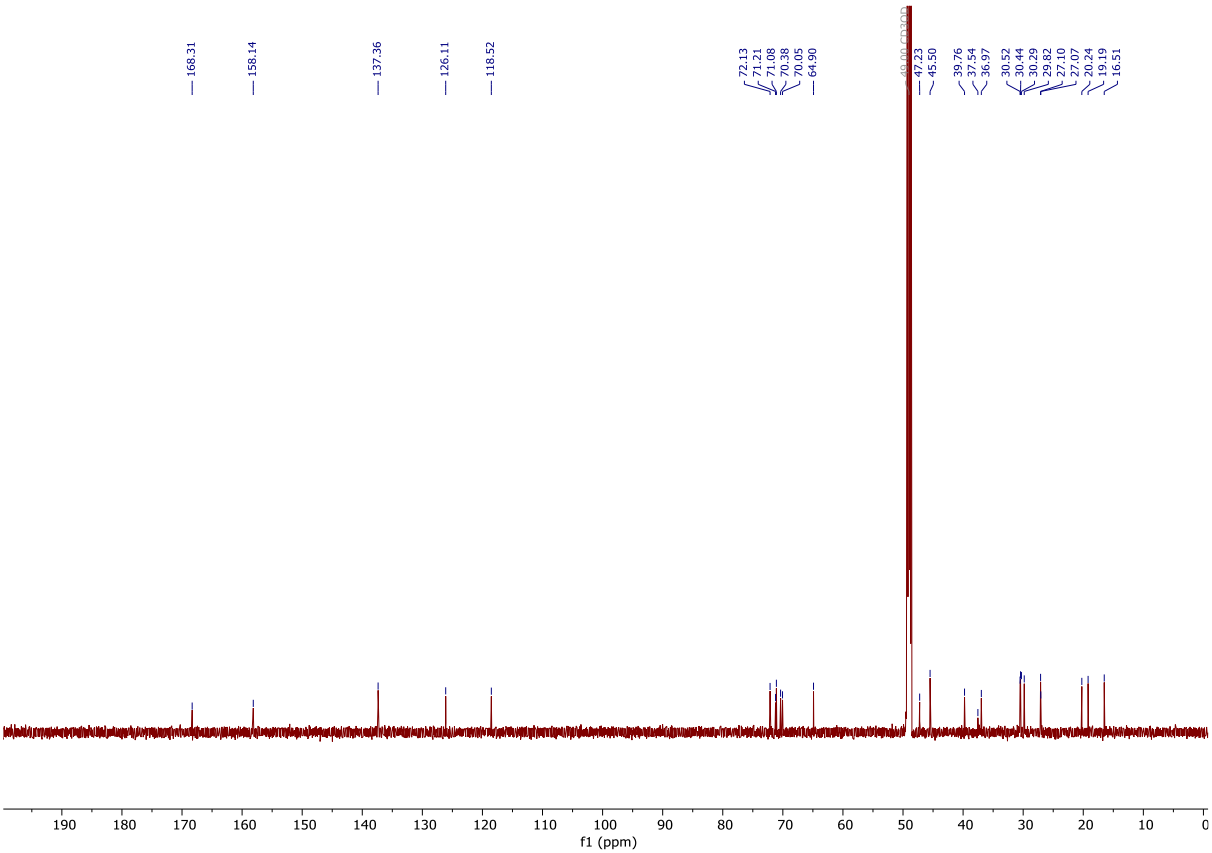

0.91
0.93
1.00
2.02
1.00
0.96
1.04
1.82
1.03
0.97
2.96
1.05
3.97
1.25
2.68
1.96
3.08
3.05
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

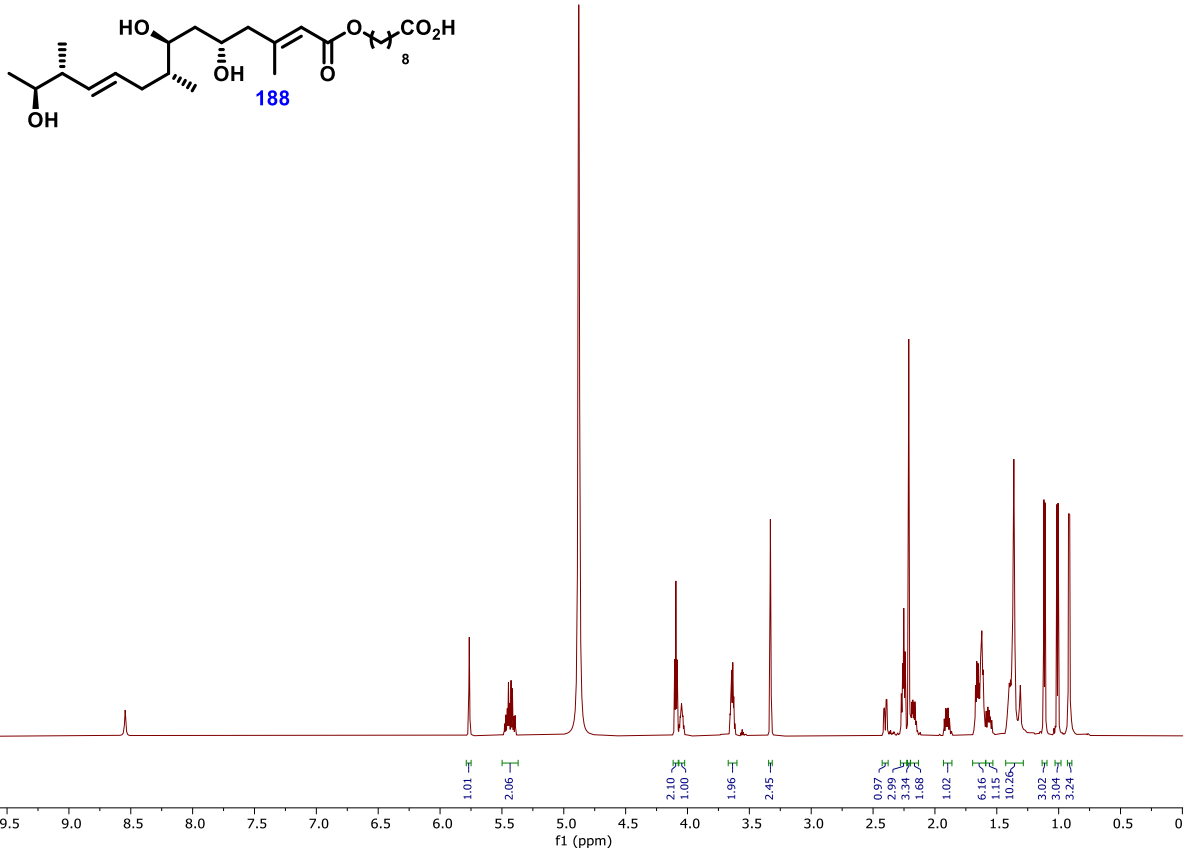

212.24
168.31
158.19
137.37
126.11
118.50
72.13
71.20
71.07
70.40
70.04
64.88
63.00
49.00 CD3OD
47.23
45.50
39.76
36.97
33.65
30.62
30.49
30.31
29.82
27.10
26.91
20.25
19.19
16.52
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

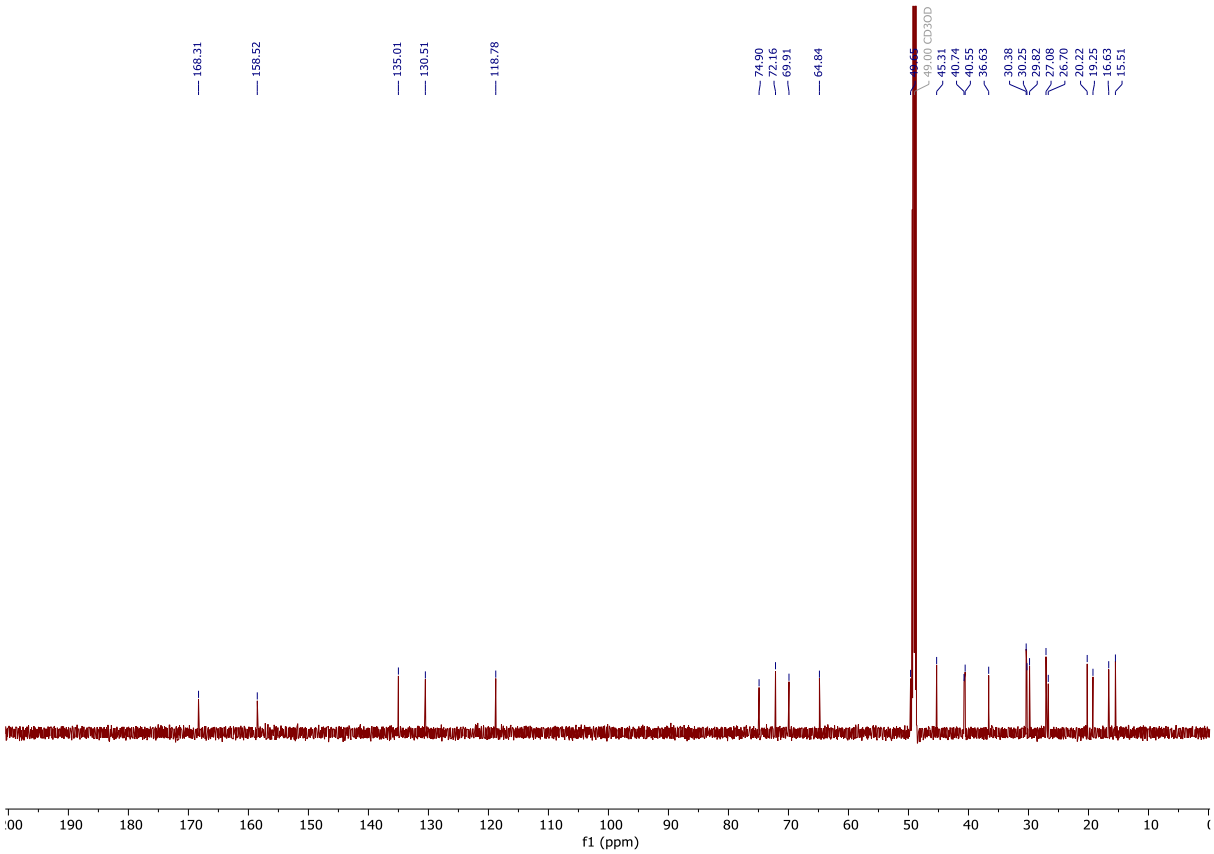

HO
O
CO2H
8
O
O
OH
OH

24

0.92
0.92
1.03
2.11
1.00
0.97
1.06
1.03
1.04
3.21
1.19
2.70
3.18
1.50
3.45
2.33
12.11
3.17
3.19
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


168.31
158.14
137.36
126.11
118.52
72.13
71.21
71.08
70.38
70.05
64.90
49.00
47.23
45.50
39.76
37.54
36.97
30.52
30.44
30.29
29.82
27.10
27.07
20.24
19.19
16.51
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


HO
OH
OH
O
O
CO2H
8
188
1.01
2.06
2.10
1.00
1.96
2.45
0.97
2.99
3.34
1.68
1.02
6.16
1.15
10.26
3.02
3.04
3.24
f1 (ppm)


168.31
158.52
135.01
130.51
118.78
74.90
72.16
69.91
64.84
49.00 CD3OD
45.31
40.74
40.55
36.63
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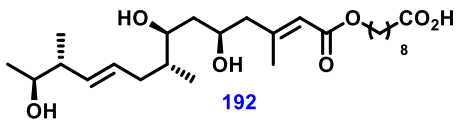
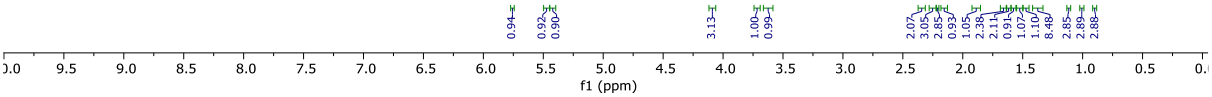
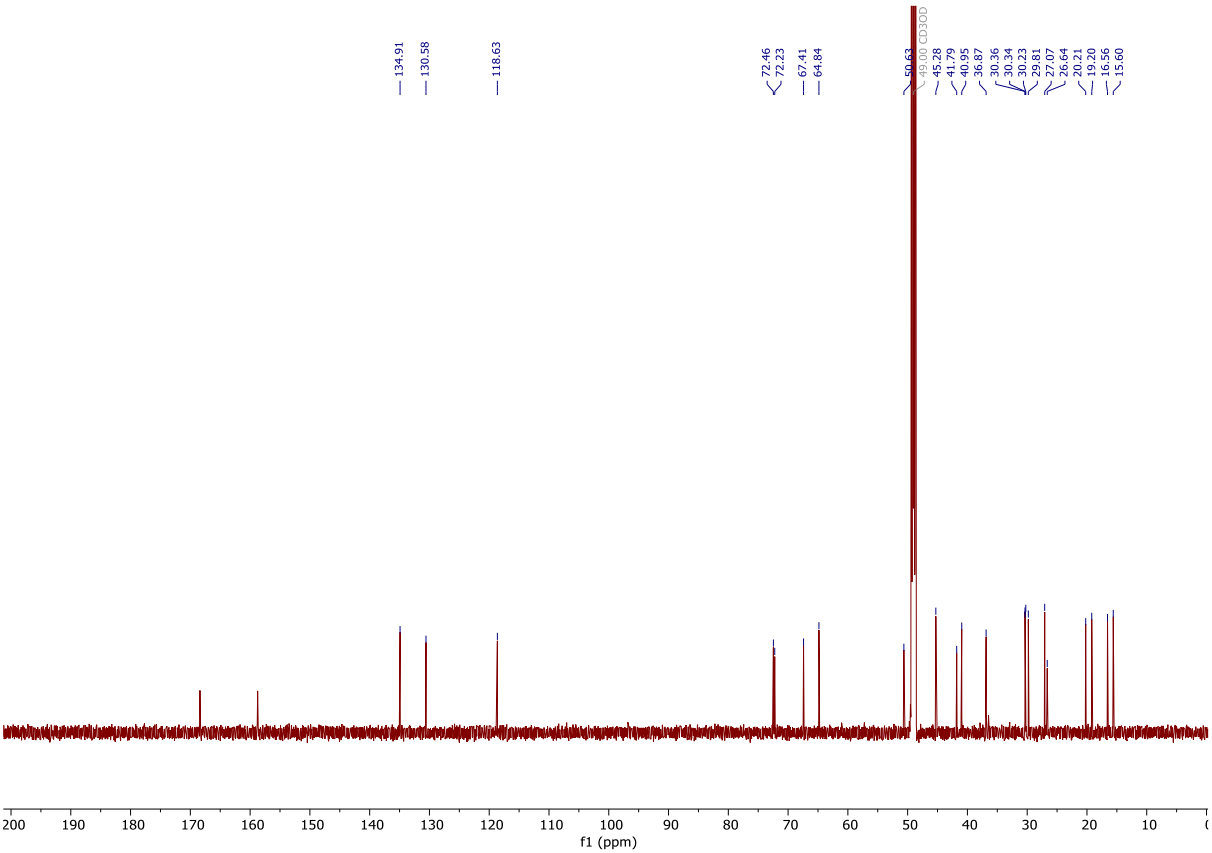
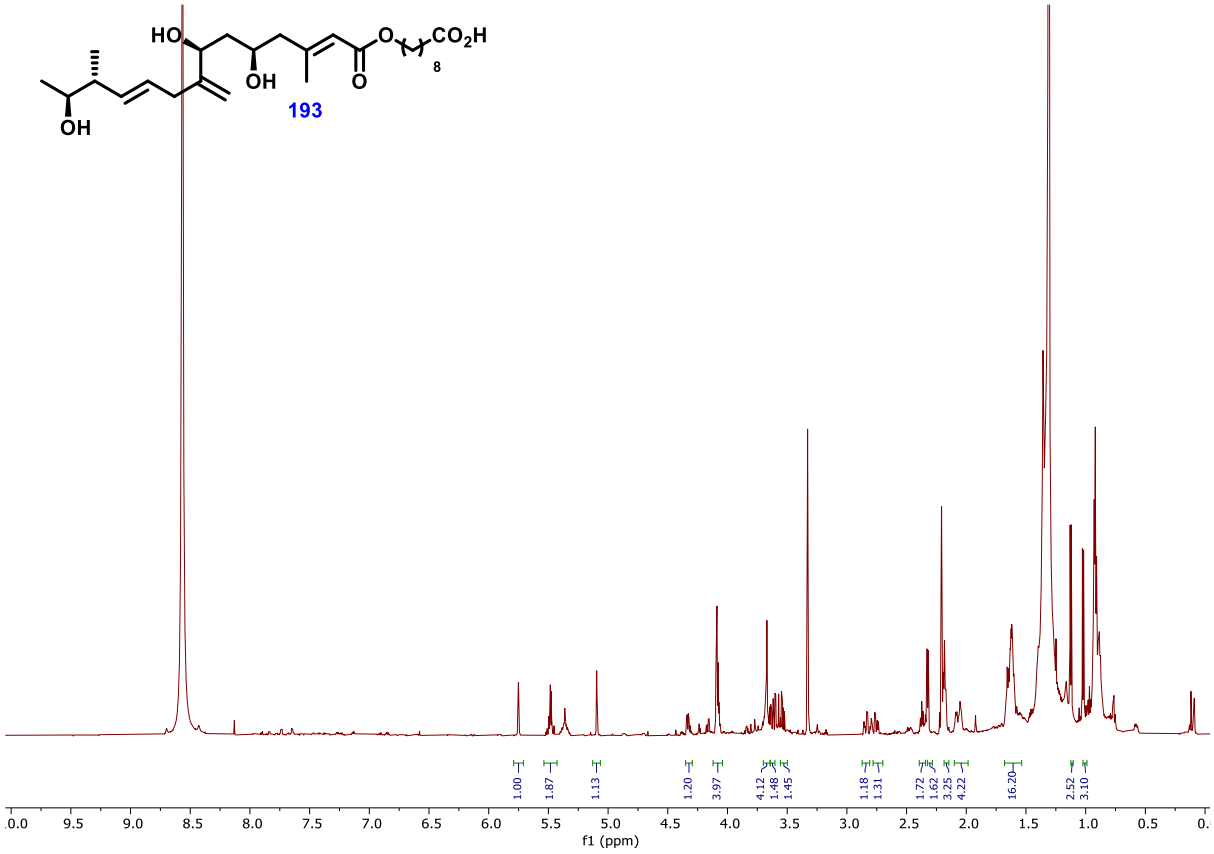
30.25
29.82
27.08
26.70
20.22
19.25
16.63
15.51
f1 (ppm)

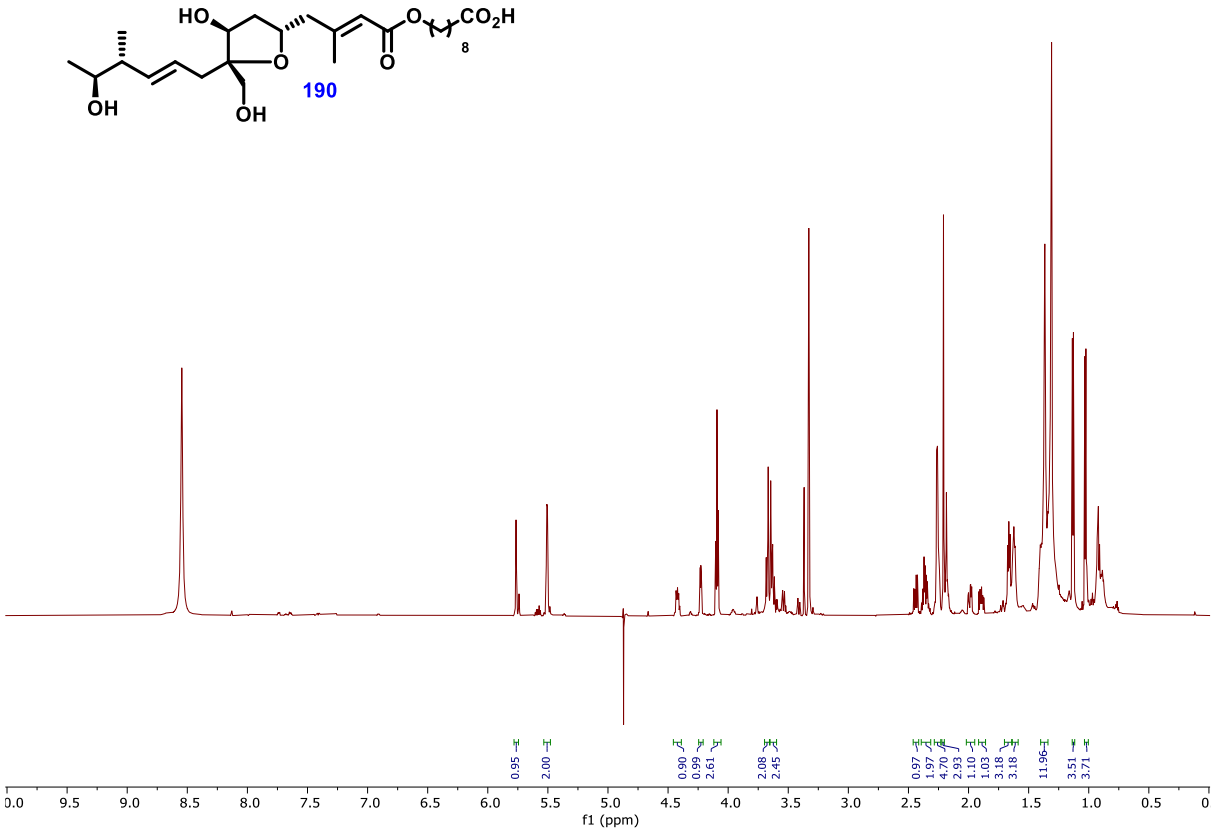

HO
OH
O
O
CO2H
8
OH

192


0.94
0.92
0.90
3.13
1.00
0.99
2.07
3.05
2.85
0.93
1.05
2.38
2.11
0.91
1.07
1.10
8.48
2.85
2.89
2.88
f1 (ppm)


134.91
130.58
118.63
72.46
72.23
67.41
64.84
50.63
49.00 CD3OD
45.28
41.79
40.95
36.87
30.36
30.34
30.23
29.81
27.07
26.64
20.21
19.20
16.56
15.60
f1 (ppm)


HO
OH
OH
O
O
CO2H
8
193
1.00
1.87
1.13
1.20
3.97
4.12
1.48
1.45
1.18
1.31
1.72
1.62
3.25
4.22
16.20
2.52
3.10
f1 (ppm)


HO
OH
OH
O
O
CO2H
8
190
0.95
2.00
0.90
0.99
2.61
2.08
2.45
0.97
1.97
4.70
2.93
1.10
1.03
3.18
3.18
11.96
3.51
3.71
f1 (ppm)